CHAPTER 4

IN VITRO EVALUATION OF CORTICOSTEROID CREAMS.

4.1. Literature Survey

Probably the most important function of an ointment base is the control that it exerts on the release of the medicament incorporated therein. For a drug to be effective, it must be first released from the ointment base. The primary requirement for topical therapy is that the drug incorporated into an ointment base must reach the skin surface at an adequate rate and in sufficient amount. Although ointment bases may not penetrate the skin to any extent nor act as carriers to transport the medicaments through the epidermal layers, there may be a marked difference in the physico-chemical properties which ultimately affect the therapeutic use of the drug.[1] The choice of an ideal vehicle for a particular drug depends not only on the physical and chemical properties of the drug but also on the components of the base as well as the conditions of the skin being treated. Physiological availability of a topically applied drug depends on both the rate of release from the vehicle and permeability through the skin. Though number of workers have attempted to correlate the physico-chemical factors with the physiological availability of the active ingredient, the role of physico-chemical factors in the overall availability of a drug has not been fully understood.

The study of the release of medicament from the bases and its passage through the skin offers a great

challenge to the research workers in the field of pharmacy as well as dermatology. The commonly used terms such as release, penetration and absorption need a clear-cut definition in the first instance because very often they are used without making any distinction.

Release refers to the availability of medicament from the base at the site of application. Penetration means the entry of the drug inside the skin whereas absorption conveys systemic distribution in the tissues of the skin. While correlating these terms it can be mentioned that better release may lead to better penetration and ultimately faster rate of absorption when the medicaments in the form of topical application are applied to the skin, the effect that is desired, is either local or systemic. Therefore, knowledge of rate and extent of release of the medicament from the vehicle is very important. The large number of bases now in use underline the need for suitable method which estimates rate and extent of release of the drug. Much information has been gained during the past twenty years but much more information is needed in order to find out a precise method for evaluating the amount and extent of release and penetration of drugs from the bases. As release of the drug from the vehicle is a first step in sequence of all steps to follow, it is advantageous to investigate the rate of release of drug from vehicle at the site of application. This necessity has given rise to introduction of numerous <u>in vitro</u> and <u>in vivo</u> techniques.

While penetration and absorption of drug in the skin is important, histological and histochemical methods and interpretation of these results are needed to measure the rate of release of the drug. The rate of release and degree of absorption of a medicament which give rise to systemic effect can be assayed by clinical effects on test animals, in which the concentrations of the drugs in blood, urine or tissues can be measured. However, pathogenic skin conditions are numerous and preclude generalisation, hence, most *in vivo* assessments are made on healthy intact skin to avoid increasing variable factors in the systems. Many authors have used chick embryo[2] for studying this aspect, however, it lacks the keratin which is present in the living skin. The other *in vivo* methods make use of animals for finding the efficiency of the bases to release the drugs and further its penetration and distribution in the body. But large number of animals would be needed to obtain a high degree of precision and meaningful results. However, these *in vivo* methods, where animals are used for studying the rate of release, may lack certain features in that the animals selected would not have sweat glands and possess more hair. Thus the skin of animals selected, may not simulate human skin. No animal is known to possess skin similar to human. Many different techniques have been developed to study percutaneous absorption or release rate *in vitro* and *in vivo*, but no single method can yield a complete picture of the complex

process of percutaneous absorption. The procedures reported in the literature may obscure rather than clarify some of the puzzles which a bioavailability study may seek to resolve[3,4].

The puzzles are :

1. What is the flux of the drug through the skin and how is this governed by the partition coefficient, the apparent diffusion coefficient, and structure-activity relationships?

2. What is the dominant route of penetration through the skin-the stratum corneum or the appendages, (pilosebaceous unit or eccrine sweat gland)?

3. Which is more important in clinical use or as a toxic hazard-transient diffusion (possible down the shunt route) or steady state permeation (usually across the intact stratum corneum)?

4. Does the drug bind to components within the stratum corneum, the viable epidermis or the dermis; or does it from a depot in the subcutaneous fat?

5. What is the rate-limiting step in the percutaneous absorption of the penetrant-drug dissolution or diffusion within the vehicle, partitioning into the various skin layers, diffusion through these strata, or removal by the blood, lymph, or tissue fluids?

6. How do skin age, condition, site, blood flow, and metabolism affect topical bioavailability? Do differences between species need to be considered?

7. How do various vehicles modify the release and absorption of the medicament?
8. Are the vehicle components truly inert or do they affect the permeability of the stratum corneum, if only by hydrating it?
9. If we need to increase the flux of the drug, should we include penetration enhancers within the formulation, on the other hand, does a too potent drug need retarding?
10. Is the formulation correctly designed for treating intact stratum corneum, thickened epidermis, or damaged skin?
11. Should the experimental design provide a full pharmacokinetic profile for the drug and so measure absorption, distribution, metabolism and excretion.

Hence, it may not be illogical to start preliminary investigation on rate of release and absorption of the drug by *in vitro* methods where the skin conditions are fairly well simulated. No doubt clinical trials would form an integral part of extended studies.

The *in vitro* methods are means of assessing the ability of the vehicle to liberate medicament under some fixed conditions of the test. The methods reported in literature have been used to give comparative data and most of them are empirical and hence results obtained from such tests are difficult to compare with each other.

The general advantage of using, for example, an excised skin technique is that the investigator may control the laboratory environment and so elucidate individual factors which modify drug penetration. Thus, *in vitro* methods are valuable for screening procedures and for deducing physicochemical parameters such as fluxes, partition coefficients, and diffusion coefficients. A theoretical disadvantage of such a technique is that the method does not exactly duplicate the behavior of living tissue *in situ*, particularly with respect to a capricious blood supply and metabolism. A minor problem with topical steroids is that they constrict the cappillaries *in vivo* and may decrease their own clearances; in *vitro* this would not happen. However, provided it can be established that the rate-limiting step in the percutaneous absorption of a compound *in vivo* is diffusion through the dead horny layer, then a well designed *in vitro* methodology should produce results which correlate reasonably well with *in vivo* studies.[5,6] The inherent assumption that the stratum corneum functions as a permeability barrier in the same way *in vivo* and *in vitro*, has been tested mainly for transepidermal water loss because of its relative ease of measurement.[7,8]

The release of the drug by *in vitro* method is essentially a study of diffusion rates, neglecting the emollient and protective properties of the base.

The methods which are included under in vitro studies are classified as under.

4.2. Release Methods (Diffusion) without a Rate-Limiting Membrane.

(a) Chemical and Physical

(b) Microbiological

4.3. Release Methods (Diffusion) with a Rate-Limiting Membrane.

(a) Chemical and Physical

(b) Microbiological.

4.2. Release Methods (Diffusion) without a Rate-Limiting Membrane

The assumption made in most of these methods is that the distribution of medicament between the base and area under treatment will be similar to the distribution between vehicle and medium of test. The distrubution of drug will depend upon the partition coefficient of the drug in oily and aqueous phases. A base in which the drug is not soluble but is only dispersed, can make the drug available only to that portion which can be leached by the extraction medium from the surface layer of the ointment or cream. For this purpose the base is required to be insoluble in the extraction medium. These procedures record the kinetics of drug release from a formulation to a simple immiscible phase which is supposed to correspond in properties with human skin. The limitations of this assumption are evident in light of the biological and physico-chemical complexity of mammalian skin. Such techniques measure drug-vehicle interactions and the

release characteristics of the formulation, and as such they are valuable but have little direct relevance to the process of percutaneous absorption.

4.2.a. Chemical and physical

Hawking[9] studied the topical release of sulpha drugs with a static method of measuring diffusion by estimating the amount of sulpha drugs from a dispersion diffusing into known surface area of agar or gelatin incorporated with acid solution of p-dimethyl benzaldehyde. The sulphonamide reacts with p-dimethyl benzaldehyde to give yellow coloured zones.

Generally the solvents which have been used include simple aqueous media such as water, agar, and gelatin[1] and isopropyl myristate, an organic solvent with a blend of polar and non-polar characteristics which make it more like skin.[10-12]

One arrangement used to study the release of corticosteroid from model ointment systems had a liquid oily composition containing betamethasone-17-valerate, floating on aqueous alcohol (representing skin) on top of a chloroform sink which simulated the blood supply.[13] A stirrer with three vanes agitated the layers, and the amount of steroid delivered to the chloroform was measured as a function of time.

A similar arrangement exposed the open neck of a glass vessel filled with a formulation to a stirred immiscible receptor phase; the orientation of the

container depends on the relative densities of its contents and the receptor fluid. Filter paper or a similar material holds the product in place and prevents bulk transfer of the vehicle to the solvent.[14,15] Sometimes a simple dialysis membrane such as cellulose acetate may close the receptacle[16, 17], or a simple bag of the membrane may be used.[18]

Patel et al.[19] used modified method by using a petridish in place of test tube, to increase the sensitivity of the colour zones produced. Locke and Sprowls[20] gave a mathematical basis using the rate of diffusion of sulpha drugs from bases into an agar gel incorporating p-dimethyl benzaldehyde (Ehrlich's reagent).

Mawking[9] used a cellulose cylinder into which gelatin was poured and then 5 ml of sulpha solution was added. The use of cellulose cylinder made possible the slicing of the gelatin in the cylinder transversely. The uncut cylinder was kept at r.t. Then 3 mm slices were cut and analysed for sulpha colorimetrically. A colorimetric method was suggested by Bandalin and Kemp[21] to assay sulpha drugs. The sulpha ointment was applied evenly around the inside of the standard test tube to which either saline or serum was added. The tubes were incubated at 37° for varying times after which the fluid was decanted and analysed colorimetrically. Surfactants markedly increased the drug release.

Fuller et al.[22] estimated sulpha drug from glycero-gelatin jelly into a stream of water moving at 20 ml hr^{-1}. The diffusion was rapid until the surface layer of the jelly was exhausted. This method is simple and elegant and, when compared with in vivo tests, claims a satisfactory agreement between in vivo and in vitro tests.

Foster et al.[23] have also used slightly modified process to find out the efficiency of Jelene base for salicylic acid. The ointments were packed in weighing bottle caps and placed in a modified rotary apparatus and subjected to constant oscillation at 120 cycles/min and analysed for acid content.

Young et al.[24] have proposed an interesting method to study the rate of release from ointment. They filled a gel containing fluocinolone acetonide in a petridish and kept it at the bottom of one litre beaker and immersed the beaker in water maintained at 37°. Isopropyl myristate 200 ml was gradually layered over the vehicle to begin the experiment and the solution was agitated with the help of stirring blade rotating just above the petridish. Aliquot samples were withdrawn and analysed colorimetrically. Dempaski[25] modified the above method while studying betamethasone ointment and the petridish was closed in a beaker in which 400 cc water was poured slowly. The supernatant liquid maintained at 37° was agitated with a stirrer at 60 r.p.m. The aliquot samples were withdrawn and analysed spectrophotometrically for the steroid content.

4.2.b. Microbiological

Since topical applications are often intended for their antiseptic value, a number of methods and techniques have been devised which depend upon a form of microbial assays. The tests which aim at assessing the antiseptic value against different organisms give an indication of the rate and degree of release of medicament from the vehicle. When different bases are used, comparison should be made only when all conditions are met, namely organism used, method of diffusion etc. which seldom is the case. One of the earliest methods was developed in 1895[26] when glass cover slips were treated with S. marcescens dried and then introduced in the ointment for fixed period of time. The slips were washed and transferred to sterile broth and incubated to see the growth of microorganism. Cheyne[27] spread ointment on cover slips which were then laid under agar in Petridish. After incubating the surface of agar with S. aureus, the zones of inhibition were observed. Reddish[28] devised the most widely used test method for the effectiveness of antiseptics included in ointment bases. The method with certain modification was eventually adopted by Food and Drug Administration of USA and remains the only official test in use. In this method the plates were incubated with a culture of M. pyogenes var. aureus and spread with the test ointments. After

incubation the width of the zones of inhibition was taken to be an indication of antiseptic value. Reddish suggested that the agar of the petridish simulated the conditions met within wound and skin. Since "it is permeable, semisolid, isotonic and constitute a valuable laboratory means of approximating the conditions found in human and animal tissues". However this is open to question because, apart from chemical and physical differences there is no cell membrane to penetrate, no keratin, no cell debris, pus, skin flora and excretory appendages all of which are known to influence the activity and absorption of medicament. The method is also limited to antiseptic preparations and is not applicable to other ointments.

Reddish and Wales[29] and other coworkers[30] using this and similar tests have shown the much greater efficiency of emulsified bases over fats and oils as a carrier for antiseptics. Even the modification of the test of Reddish in which a weighed quantity of ointment was spread over a definite area, indicated the efficiency of e/w bases over oils, fats and water.

Pillsbury, Livingwood and Nichols[31] proposed a technique in which the hands were scrubbed in a standard way and the number of organisms removed was estimated by colony counting on agar base after incubating with the rinsings. Ointments were rubbed on each

hand and forearm, allowed to remain for a stated period of time, removed and after washing again the number of organisms remaining was estimated. Comparison of dimunition in the count was the criterian used for evaluating the efficiency of bases. The objections to such a method are numerous. The main criticism is that it involves too many variables to be of practical value as a reliable technique. An agar tube method was developed by Billups et al.[32]

4.3. Release Methods (Diffusion) with a Rate-Limiting Membrane

The use of a membrane composed of cellulose film is an attempt to simulate in the in vitro studies the barrier presented by the skin to the topical application. Both artificial and natural membranes have been used and the assumption is made that the process of penetration in the skin is similar to the quantitative diffusion through a membrane[33]. This, of course, will not be very similar to the skin which presents a more complex system both physically and chemically.

4.3.a. Chemical and Physical

(i) Simulated skin membrane :

Because human skin may be difficult to obtain and varies in its permeability, many workers used other materials to simulate it.[34] However, in most circumstances the membrane simply hinders the penetrant as it diffuses through its channels and the transport process correlates at best with molecular permeation

across porous capillary endothelium; the transfer mechanism is, dialysis or passage through macroscopic ducts filled with solvent[35,36]. Evidence that diffusion may occur mainly through hydrated regions of Co (Polyether) polyurethane membranes has been presented.[37]

Rae[38] incorporated sodium chloride into various ointment bases which were then introduced individually in a glass tubing to one end of which was attached a cellulose film membrane. The ointment was gently forced to the membrane end of tube and this was immersed in distilled water for 24 hr. The chloride diffusing out was analysed by using silver nitrate. He stated that the various results obtained probably represent what takes place when ointment is applied to broken skin.

Some workers[39-42] used polydimethylsiloxane membranes for drug transport studies because they are hydrophobic, relatively highly permeable, and are easy to prepare.

Patel[43] studied separately, by using different cells, the release, up-take and permeation behaviour of salicylic acid. The silicone rubber membrane was used as a barrier and the cells were agitated on shaker kept in incubator at constant temperature. The aliquot samples were removed from the other side of the cell and analysed.

The commercial product may contain some 20 to 30% of a dispersed phase of fumed silica Filler[44] which provides an obstructive effect to diffusion, thus, membranes from a single batch should be used throughout an experiment. Lovering and Black[45] investigated the diffusion layer effect which operated during the passage of phenylbutazone through polydimethylsilioxane and showed non-Fickian behavior. Koran and Huyak[46] used a method suggested by Izgu[47] in which diffusion from ointment in a hollow cylinder placed in a centre of filter paper moistened with indicator solution was estimated by measuring the distance of the outer edge of indicator ring on the paper. Makano and Patel[43] used silicone rubber and reported that their results agreed with the in vivo data of Stolar et al.[48]. Whitworth et al.[49] had suggested a method in which the ointments were packed in hollow plastic stopper, one end of which was closed later by using a membrane and the whole immersed in water. The samples were withdrawn from aqueous solution and analysed by chemical method. Membranes may also be obtained from biological materials, such as collagen[50]. A promising approach uses egg shell membranes, since like the stratum corneum they consist mainly of Keratin. For salicylic acid permeation at various pH values, this membrane behaves as a dialysis medium similar to cellulose acetate; treated with isopropyl myristate to simulate the lipid phase of the horny layer, the system acts like a polyamide lipoid membrane.[51]

4.3.a.2. Natural skin membranes.

Excised skin from a variety of animals, including rats, mice, rabbits, guinea pigs, hamsters, pigs, hairless dogs, and monkeys, have been used in diffusion cells. However, mammalian skin varies widely in characteristics such as stratum corneum thickness and the number density of sweat glands and hair follicles. Cattle and sheep develop skin which is even more dissimilar to that of man. Thus Pitman and Rostas[52] speculate that in these animals the bulk transport of neutral molecules of small to medium size is via skin appendages and that the composition and properties of the sebum-sweat emulsion associated with the skin is important. If at all possible, investigative problems should not be made more complex by selection of an animal tissue to represent human skin.

Astley and Levine[53] stated that storage for upto 6 months at -20° leaves human skin permeability unaffected. However, Swarbrick et al.[54] advise that, for chromene acids used as penetrants, frozen samples of excised skin should be avoided since the extent of permeation exceeds that obtained with fresh skin.

A typical procedure used to store autopsy strips of abdominal skin at -24° in heat-sealed, evacuated plastic bags. When required for an experiment, excess fat was trimed off and stratum corneum was removed by the heat treatment method of Kligman and Christophers.[55]

Epidermal sheets may also be obtained from 1 to 2 day old mice by subcutaneous injection of exfoliative fractions derived from culture supernatants of certain phage group 2 Staphylococci. Two hours later, the animals are killed and upper epidermal sheets may be rubbed off.(56,57)

Nearly all investigators clamp such membranes in a diffusion cell and measure the passage of a compound from the stratum corneum side through to a fluid bath. However, it is important that the tissue be equilibrated with receptor solution before it is fastened in the cell. If it is mounted dry and then donor and receptor solutions are applied, the stratum corneum hydrates and swells and the shunt route may constrict. This closure would lead to erroneous results for experiments which measure the permeation of drugs entering via this pathway.

4.3.a.3. Diffusion cells.

Zero-order steady state flux: A well-stirred donor solution at constant concentration delivers penetrant across a membrane to be received by an agitated "sink" receptor liquid which may simulate the blood supply in skin. The receptor fluid can be water, saline, buffer or, for poorly water-soluble compound, 50% ethanol in water. In light of biological variability of skin, it is permissible to have upto 10% depletion of the donor phase and a similar level of build up in the receptor phase (provided that saturation is not approached) Zero-

order flux conditions are not significantly violated.

Billups et al.[32] modified the method suggested by Jurist[33] where they had used a dialysis cell method for measuring the ion exchange capacity of resin incorporated in ointment base. This method was modified and used successfully by Mutimer and other workers[58]. In this method the apparatus consists of plastic components having diffusion cells using cellophane paper as membrane. This membrane separated the ointment from the aqueous sodium citrate solution in the cells which were placed in constant temperature bath. The samples from the cells were withdrawn at definite time intervals and pH was determined. Similarly a cell has been designed to measure diffusion of medicament. The cell consists of two Lucite plastic compartments separated by a cellophane membrane. The medium containing drug was placed in one compartment and diffusion occured in the other compartment which contained same vehicle without drug.

Perspex diffusion cells similar to those of Patel and Foss[59] when used with a readily available synthetic membrane such as cellulose acetate, can have large compartment and membrane areas[35].

Garret and Chemburker[60] modified a design of Lyman et al.[61] so that membranes closed both ends of a T-Joint

cell. The cell was filled with penetrant solution recycled from a reservoir and was inverted in a beaker containing receptor medium.

Bettley[62] built an immersible cell which rotated about a central axis like a wheel. As it revolved, an air bubble rushed through each chamber producing turbulence and so mixed the fluids without imposing a mechanical strain on the epidermis.

4.3.a.4. Diffusion cells :

Simulation of in vivo conditions: In general, diffusion cells designed to mimic topical therapy use a stirred or flow-through receptor solution to correspond to the blood supply and an unmixed donor phase to represent a formulation applied to the skin. The material may be a solid deposited from a volatile solvent, a liquid, a semisolid (ointment, cream, paste, or gel), a film, or even a drug device. The donor compartment may be closed or open to ambient conditions or to a controlled humidity, and additional materials may be added or the skin washed during a permeation experiment.

In man, a dermatological product may not be a steady source of penetrant, as the drug concentration may increase as solvents evaporate, decrease as the chemical diffuses, or material may be rubbed off, washed off, or shed with desquamating scales.

Alternatively a patient may apply replicate dose to the same area of skin. Some workers of Syntex Research used the improved version of the simple cell of Samitz et al.[63]. One minor problem with their design was the relative fragility, i.e. uneven pressure through the screws readily shatters the glass. A comparable design was used by Bronaugh et al.[64].

One experimental difficulty encountered with such cells is the repetitive nature of multiple sampling and receptor replacement. Cooper[65] solved this difficulty by designing the stainless steel cell in which flat surfaces provide a pressure seal for the skin after the two compartments are bolted together. Many other designs exist, including U-shaped glass vessels with skin draped over one arm[66] and modifications in which glass rods recessed in a Teflon washer support the delicate membrane[67].

4.3.b. Microbiological :

The rate of release of penicillin[68] from various bases placed in cellulose film bags immersed in saline was estimated for activity by FDA cup-plate method. Clark and Davis[69] modified the agar cup-plate method and observed on the basis of zone of inhibition that, phenyl mercuric nitrate and proflavine sulphate have shown an interesting release from the ointment bases.

The above brief review of different methods indicates that there is no single method available, which gives the correct picture regarding release, penetration and absorption of the drugs from ointments. Hence a suitable *in vitro* method, which would simulate skin conditions was adopted for study of drug release from newly formulated ointment bases.

4.4. Preparation of Creams.

Procedure :

The oily ingredients and the oil soluble ingredients were weighed and transferred in a glass beaker. The ingredients were heated together on a water bath at 80 to 85°. As soon as the clear solution was obtained, half the quantity of drug (Micronized Powder) was added to it and dissolved at 85°. The temperature maintained at 85° (oily phase).

The water soluble ingredients along with purified water were weighed and transferred in another glass beaker. The ingredients were dissolved by heating the mixture on a water bath at 80 to 85°. The temperature maintained at 85° with constant stirring (Aqueous phase).

The oily phase was slowly transferred to planetary bowl containing homogenizer at 80-85°, by straining through nylon cloth or double folded muslin cloth. The aqueous

phase was slowly added to it at 80-85°, by straining through nylon cloth. Along with homogenizer, propeller mixer was also used simultaneously for emulsification. The homogenization and agitation continued for 30 minutes while maintaining the temperature at 80-85°. Homogenization and agitation were continued until the batch cooled down to 50-52°. Sufficient warm purified water was added to the above batch to make up for loss due to evaporation. Homogenization and agitation were stopped and the stirring was started with planetary mixer blades at slow speed until the mass congealed. Remaining half quantity of drug (micropulverized) was mixed with above batch in geometrical proportion and the whole batch was passed through tripple roller mill twice.

Based on our experience, the following precautions are recommended to be observed.

(1) Emulsify oil and water phases at approximately the same temperatures (80-85°).

(2) Homogenizer must be used for proper emulsification of the oil and water phases, at a temperature of 80-85°.

(3) All the creams may be monitored microscopically to evaluate the size of the dispersed oil globules and emulsion quality of the creams during the emulsification step, prior to the congealing phase.

(4) Avoid incorporation of air into base during congealing phase and cooling phase.

(5) Use suitable straining cloths such as Nylon or other lint-free materials to strain the aqueous and oil phases.

4.5. Drug Release Studies

From the literature survey, it is clear that though during the past half century many indigenous methods have been proposed, none is considered ideal. The *in vivo* methods are usually limited by the non availability of large number of experimental animals which are frequently needed to yield meaningful results. Perhaps a major difficulty in attempting to develop a procedure for evaluating absorption lies in the fact that no animal possesses a skin quite the same as that of man. Therefore, any correlations which could be made from these experiments are necessarily limited.

From the review it is also clear that there is no ideal *in vitro* method which perfectly simulates skin conditions. However, the methods suggested by Young[24] and Dempaski[25] seem to be more practical, but these also do not simulate the skin conditions perfectly, as in both these cases the naked ointments without any membrane were exposed to the surrounding fluid in the beaker. Moreover the ointments were submerged under water which was just the reverse of actual skin conditions. Hence the above two methods were modified to make them more suitable for topical products. It was felt that the modified procedure given below may have some significance as it simulates the practical conditions of application of ointments on the skin. This is in fact similar to Dissolution Test Apparatus USP which simulates the conditions of stomach.

An attempt has been made here to see that the conditions which are maintained when the ointment is applied. For this purpose the ointment is spread on the membrane which is later allowed to hang in the aqueous dissolution medium so that the upper surface of the ointment is exposed to air as is the case in actual practice, when the ointment is applied to the skin.

4.5.a. Experimental procedure

FIG 5-1 : In Vitro Drug Release Apparatus.

About 5 g of BV, TA, HAL and 12 g of FA Creams under test were spread on the cellophane paper attached to hollow glass cylinder open at both ends, and having

5 cm internal diameter. To the one end of the cylinder the cellophane paper with cream spread on it was tied with the help of rubber band. This cylinder was suspended with clamp, with cellophane paper downwords in a beaker. Distilled water (200 ml) was added to the beaker which was kept in constant temperature water-bath, maintained at 37±1°. While suspending the cylinder care was taken to make sure that just 5 mm height of the cylinder with the membrane, spread with the creams was immersed in the medium. The water in the beaker ie. the solution, was agitated with the help of magnetic stirrer, driven by the instrument. The speed of the stirrer was adjusted to 100 rpm with the intention to stir the solution and allow the released drugs to get properly distributed in aqueous medium.

At suitable intervals, 20 ml of the sample solution was withdrawn from the beaker, and was replaced by an equal amount of fresh distilled water also maintained at 37±1°. The drug in the sample solution was extracted thrice with 10 ml of chloroform each time. For the better separation of layer 1 g of anhydrous sodium phosphate was added during extraction. About 25 ml of chloroform layer was evaporated on the water bath at 65 to 70°. Remaining amount of chloroform was allowed to cool at room temperature and analysed for corticosteroid drugs colorimetrically. Photograph of the apparatus is dipicted in figure 5-1.

TABLE 5-1 : Cumulative Percentage Release of Triamcinolone Acetonide from Creams.

Cream Nos.	Time (hr)	Cumulative Percentage Release*				
		1	2	4	5	7
1		6.09	9.01	14.20	15.64	17.71
2		1.00	2.11	4.40	5.15	6.63
3		1.12	2.36	4.72	5.29	6.57
4		0.30	0.96	-	2.46	3.49
5		5.00	7.62	10.83	12.14	14.86
6		1.18	1.94	4.89	5.53	7.28
7		2.90	3.96	7.23	8.32	10.17
8		0.83	2.57	4.98	5.72	7.69
9		1.24	2.72	4.80	5.70	7.36
10		0.65	1.54	3.09	-	4.48
11		0.22	0.94	-	1.70	1.99
12		0.89	2.26	4.43	5.35	7.44
13		3.97	7.32	11.52	13.69	17.24
14		1.00	2.28	4.23	5.34	6.28
15		0.41	1.40	-	3.21	4.44
16		1.36	2.32	3.82	4.38	5.42
17		1.00	1.64	3.87	5.03	5.90
18		0.36	1.40	3.22	4.23	5.32
19		1.00	2.29	4.66	5.66	7.27
20		0.41	1.75	-	3.24	4.43
21		0.59	1.65	3.40	3.95	5.26
22		0.36	1.70	4.12	4.96	6.21
23		1.00	1.99	3.74	4.81	5.93
24		0.24	1.44	-	3.68	4.89
25		4.91	7.30	10.90	12.02	14.71
26		1.66	2.78	-	5.13	6.13
27		6.69	10.26	15.32	17.00	18.44

* Average of two readings.

FIG. 5-2

RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

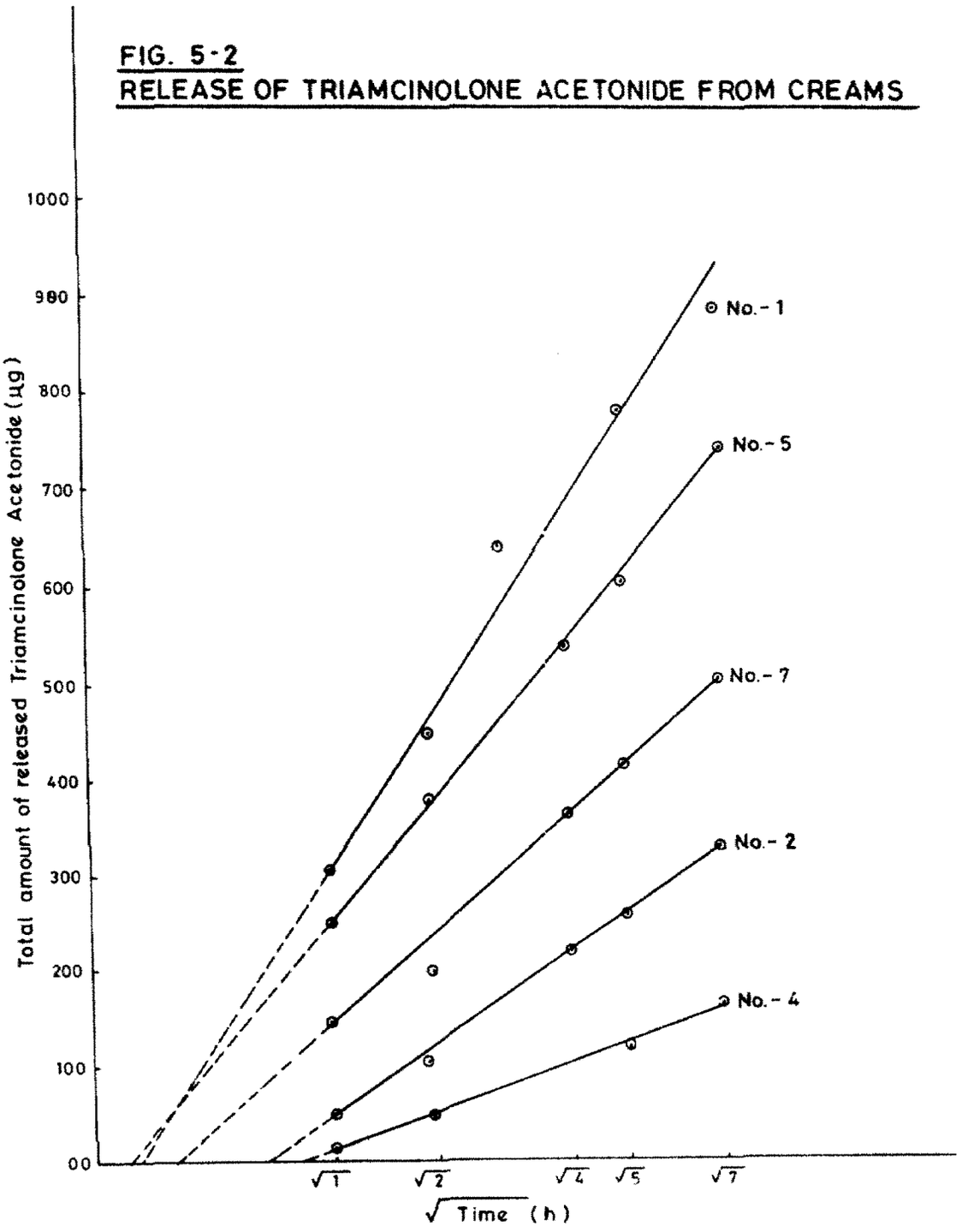


FIG. 5-3
RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

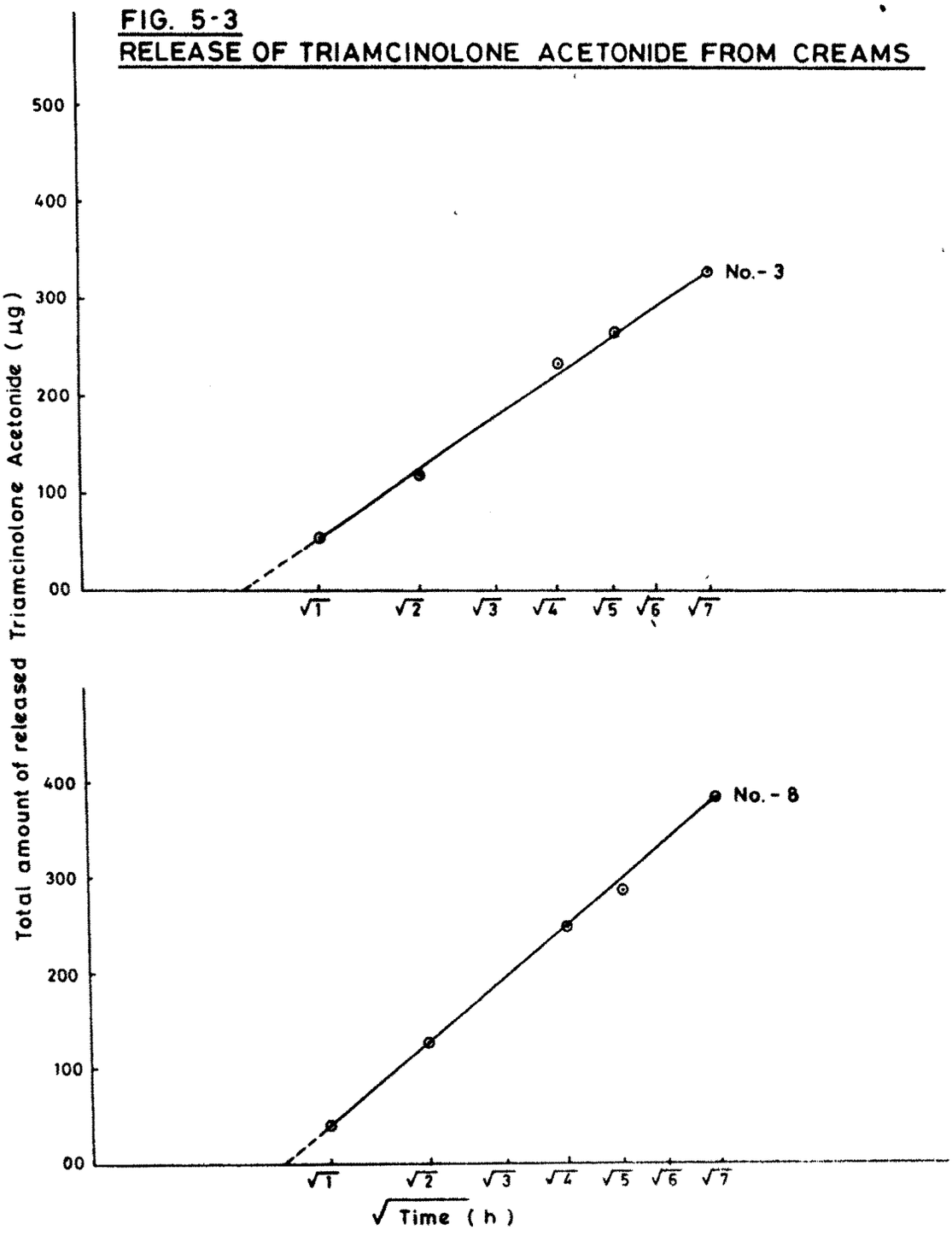


FIG. 5-4
RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

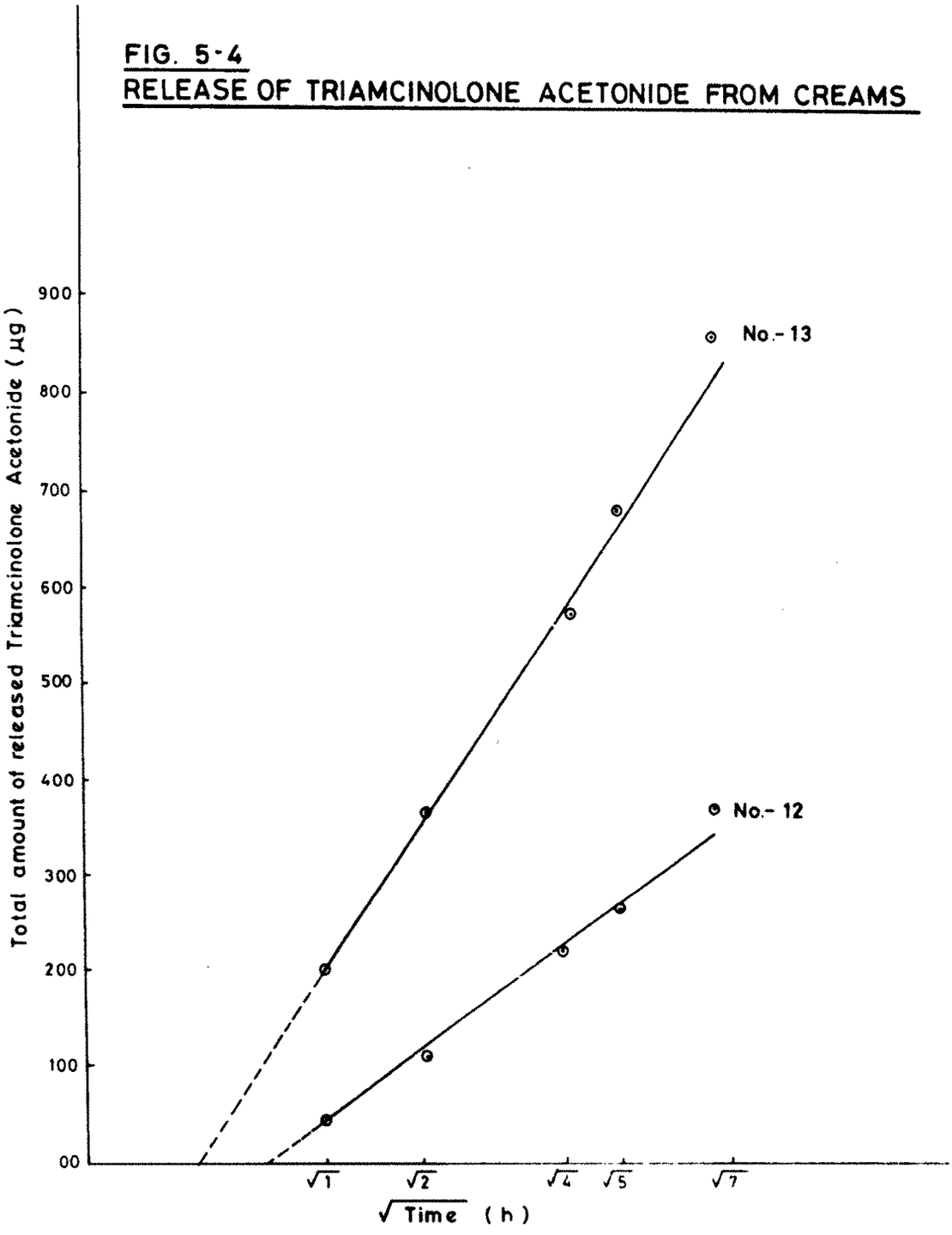


FIG. 5-5

RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

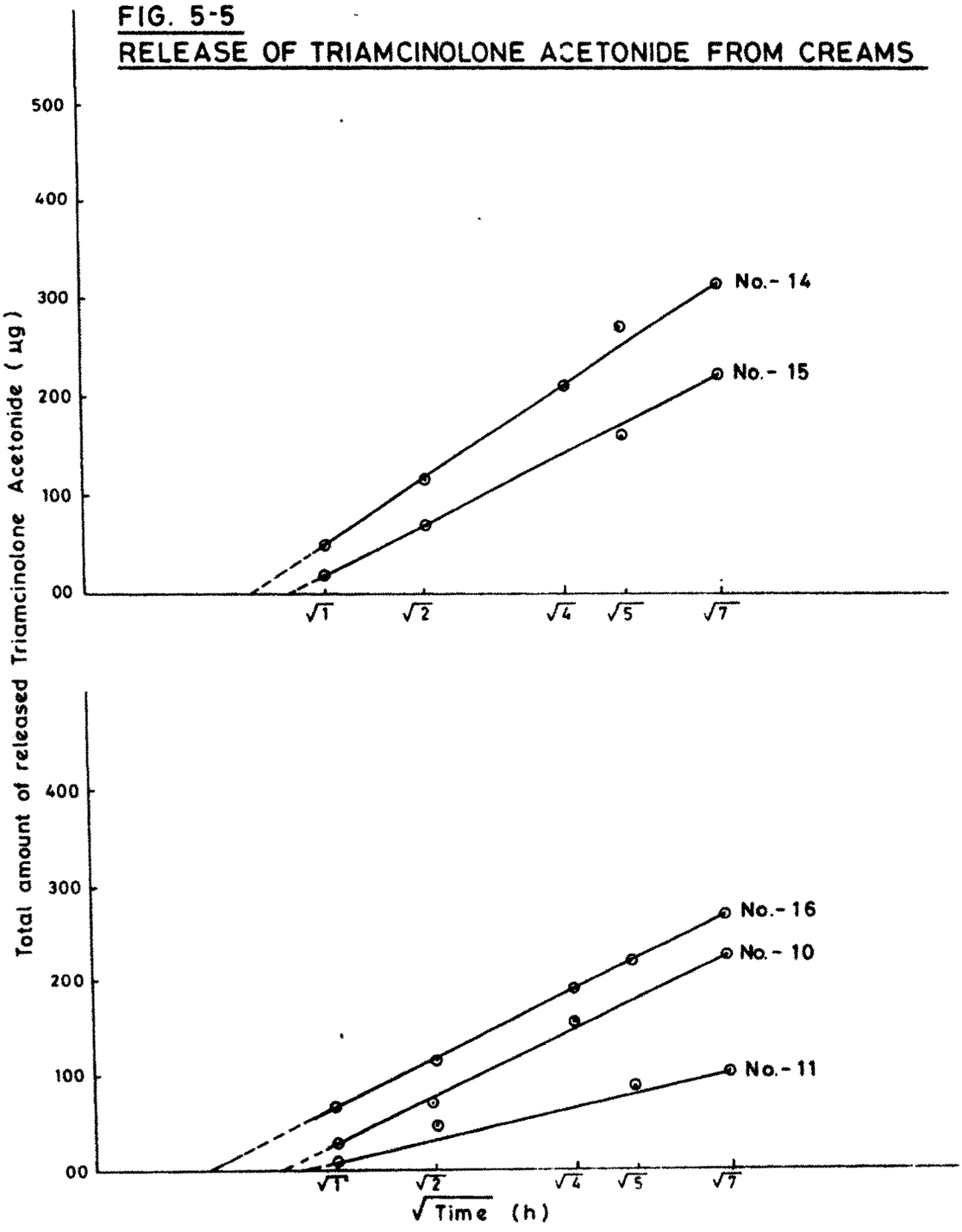


FIG. 5-6

RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

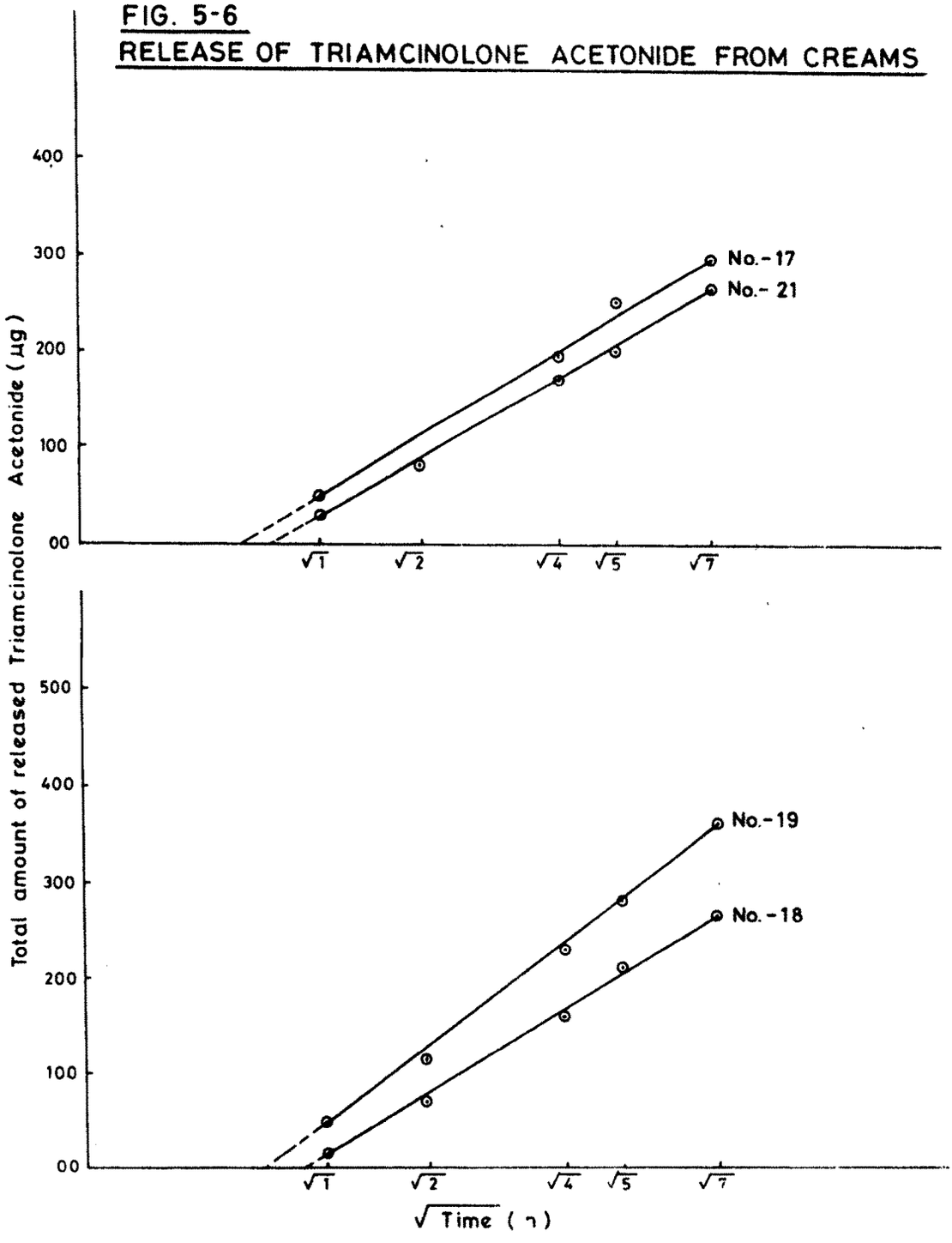


FIG. 5-7

RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

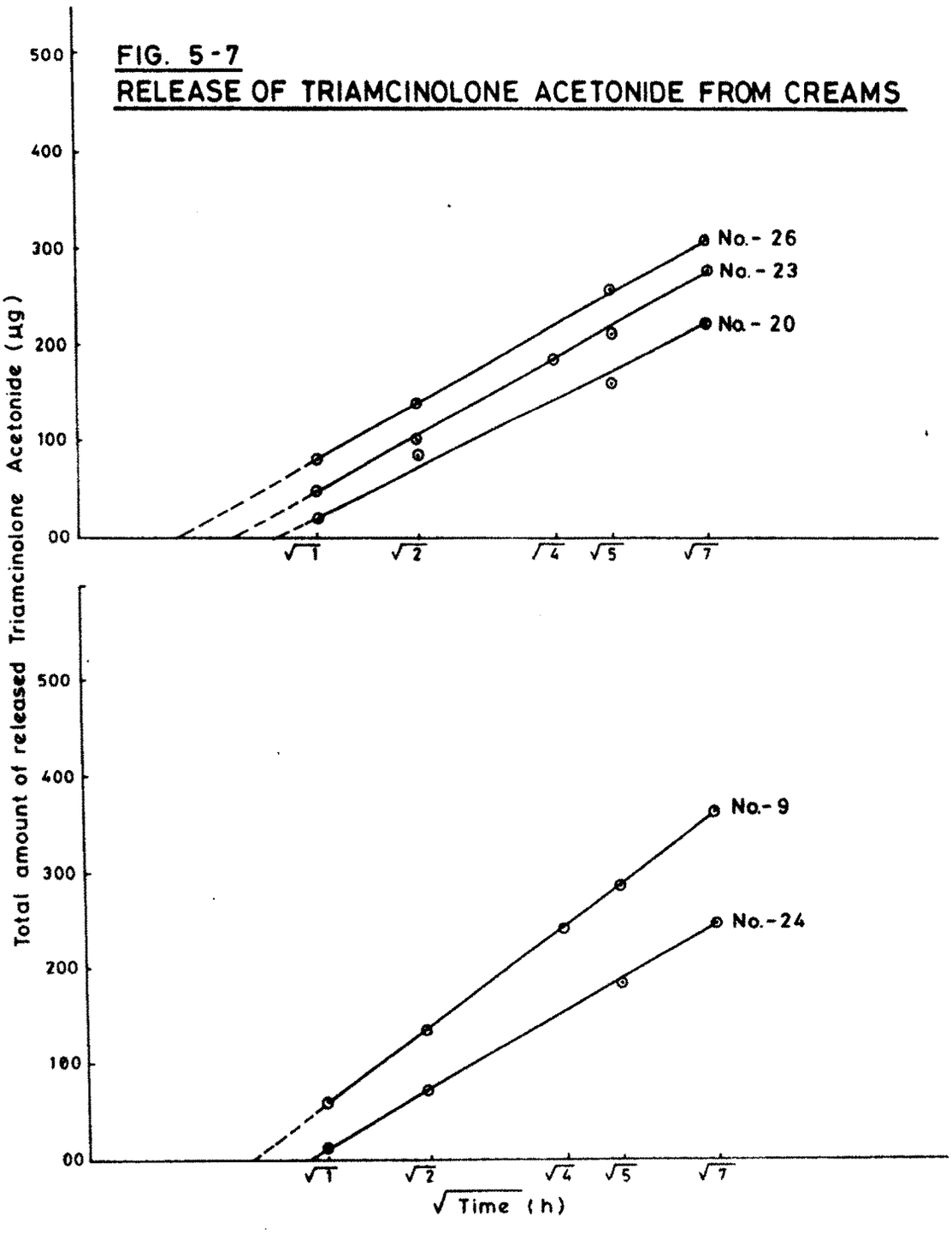


FIG. 5-8

RELEASE OF TRIAMCINOLONE ACETONIDE FROM CREAMS

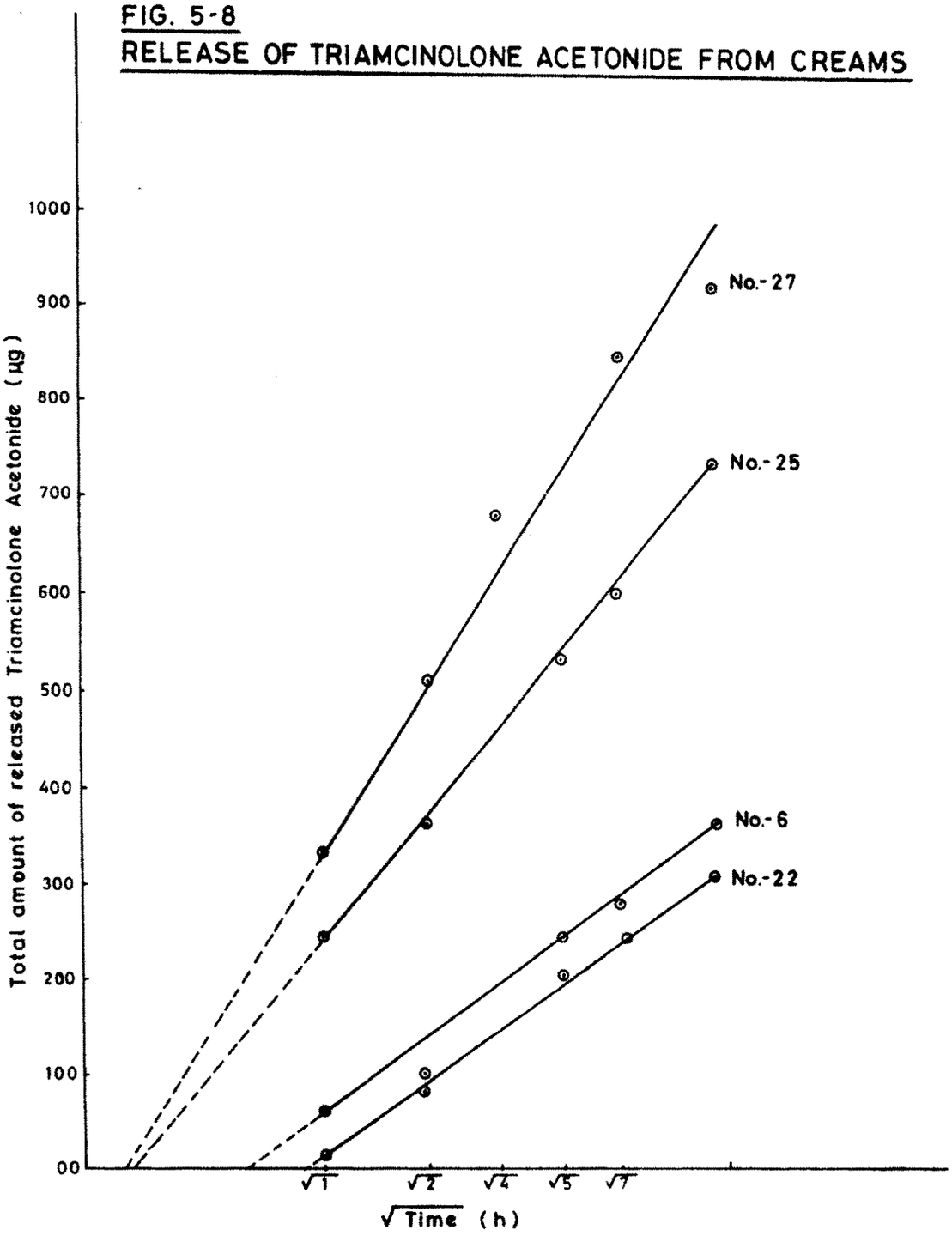


TABLE 5-2 : Cumulative Percentage Release of Betamethasone 17-valerate from Creams.

Cream Nos.	Time (hr)	Cumulative Percentage Release* 1	2	4	5	7
1		4.03	7.53	12.54	14.36	17.78
2		0.13	1.02	-	2.79	4.03
3		2.22	4.04	7.37	8.78	10.77
4		0.86	1.68	-	4.00	5.63
5		4.70	7.26	10.63	12.29	15.24
6		2.21	4.24	6.83	7.72	9.56
7		3.05	4.94	7.77	9.36	11.02
8		1.99	4.18	7.10	8.20	9.46
9		0.60	1.67	-	2.97	3.76
10		0.94	2.24	4.10	4.74	5.79
11		NIL	NIL	-	0.53	1.47
12		2.87	3.95	7.30	8.69	10.53
13		4.22	6.84	10.93	12.75	15.39
14		2.00	3.65	5.81	6.41	7.75
15		NIL	NIL	-	0.81	1.37
16		0.40	1.12	3.47	4.30	5.44
17		0.54	1.13	3.88	4.76	5.97
18		0.34	0.91	-	3.99	5.30
19		0.40	1.85	3.88	4.47	5.72
20		0.34	1.64	-	4.05	5.55
21		0.54	1.80	3.59	4.16	5.27
22		0.94	1.64	-	3.11	4.13
23		0.34	1.11	-	3.19	4.26
24		0.27	0.70	-	2.15	3.37
25		2.01	3.96	7.46	8.56	9.75
26		NIL	1.23	-	3.33	5.31
27		6.58	8.94	13.62	15.37	17.59

* Average of two readings.

FIG. 5-9

RELEASE OF BETAMETHASONE 17-VALERATE FROM CREAMS

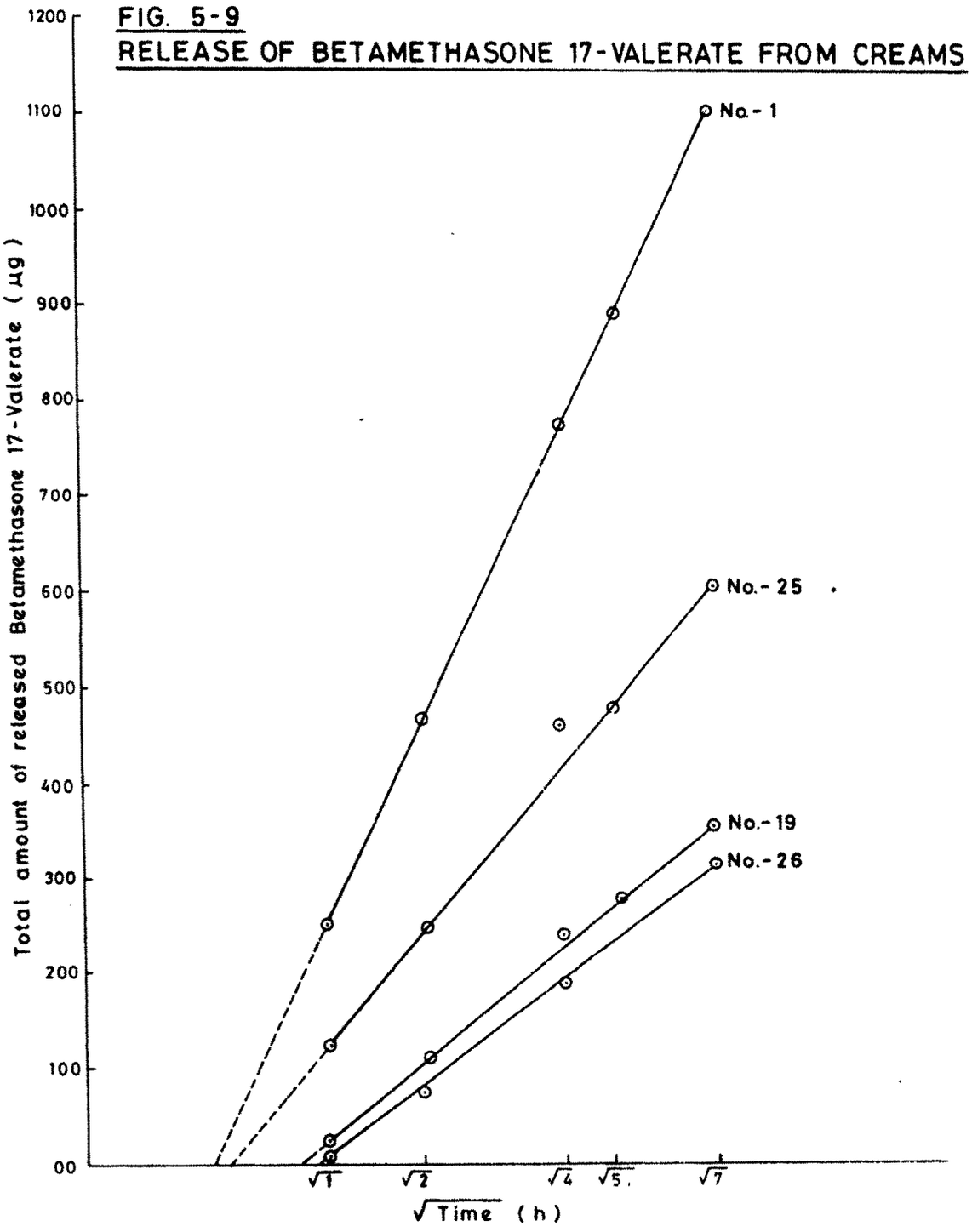


FIG. 5-10
RELEASE OF BETAMETHASONE 17-VALERATE FROM CREAMS

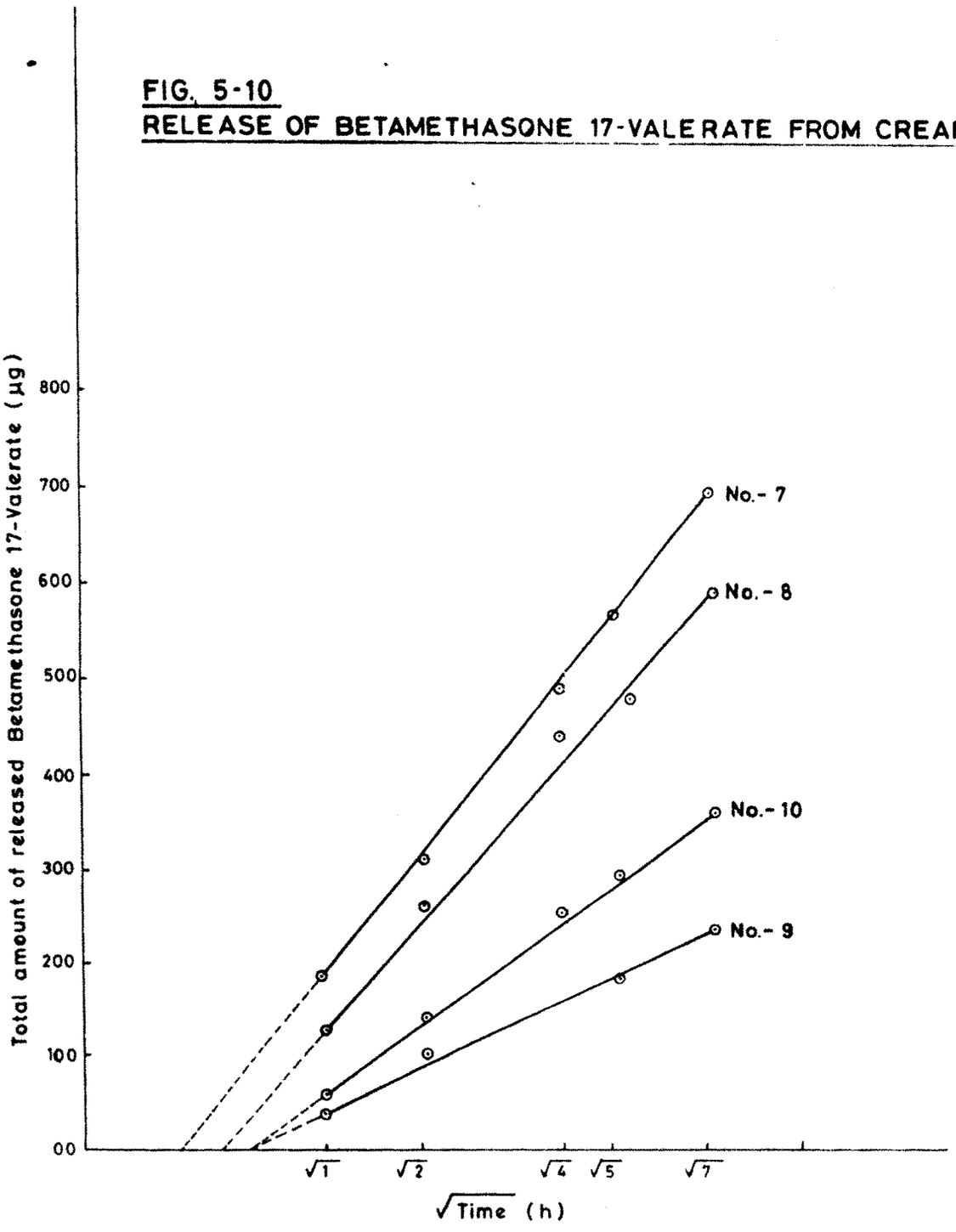


FIG. 5-11
RELEASE OF BETAMETHASONE 17-VALERTE FROM CREAMS

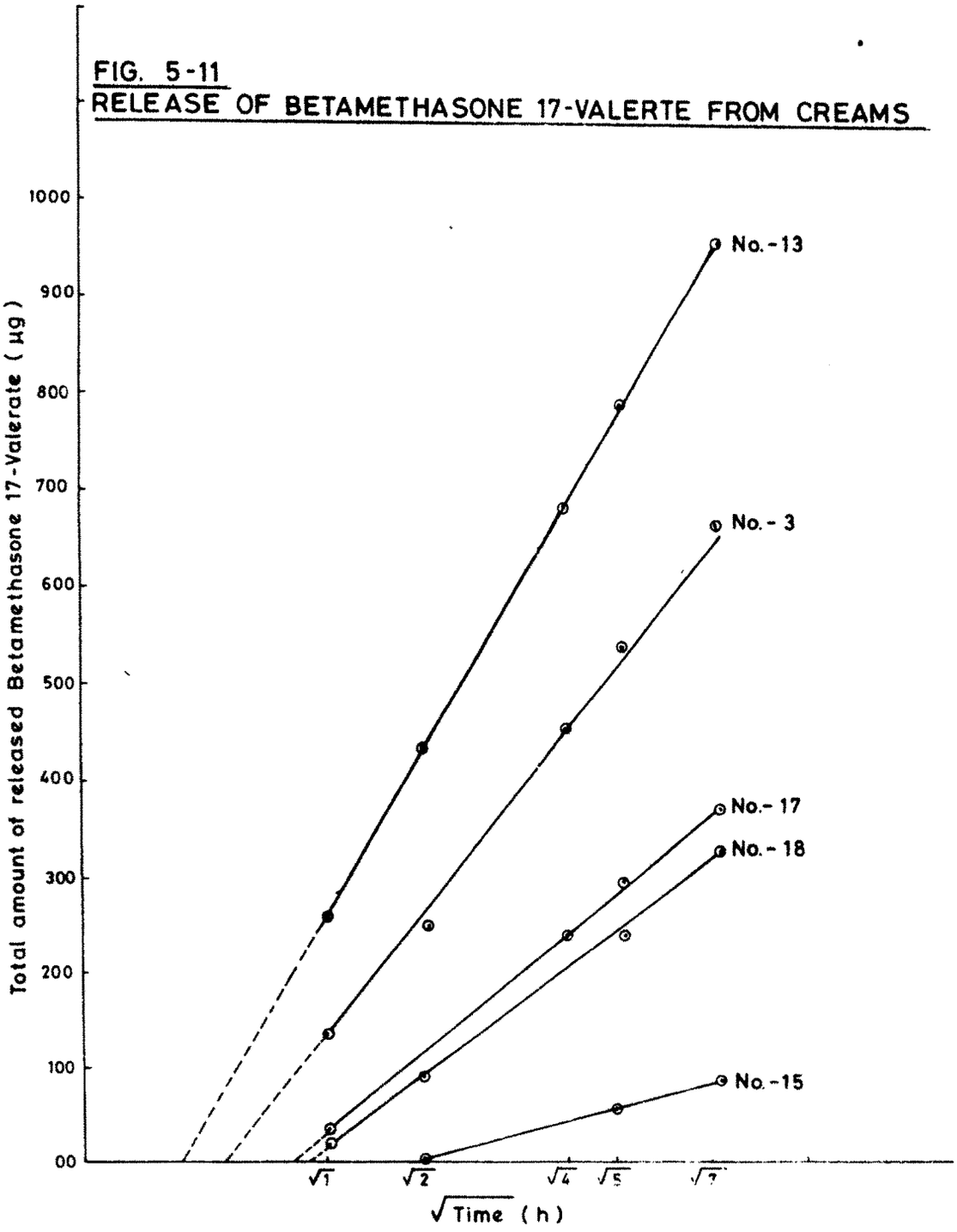


FIG. 5-12

RELEASE OF BETAMETHASONE 17-VALERATE FROM CREAMS

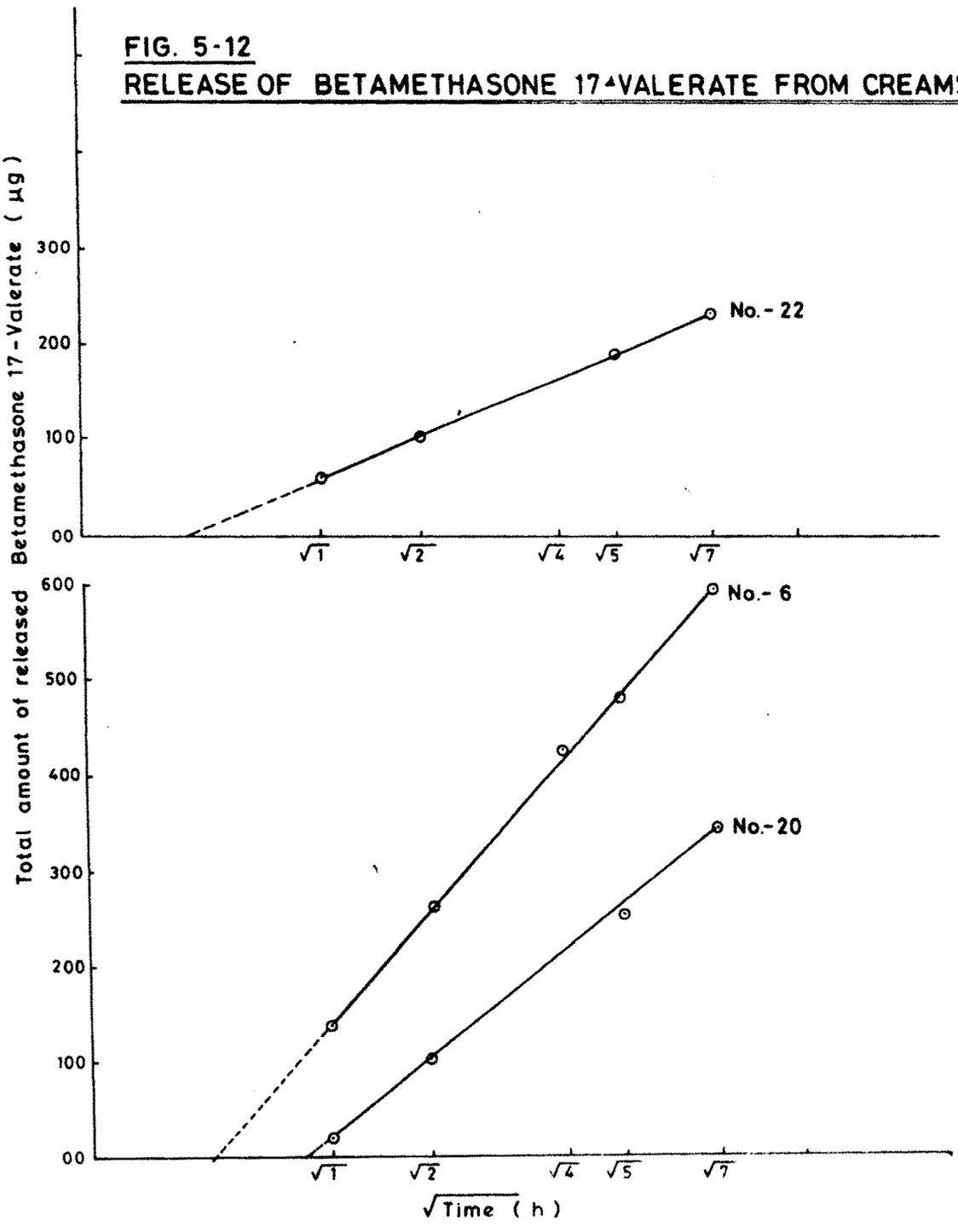


FIG. 5-13

RELEASE OF BETAMETHASONE 17-VALERATE FROM CREAMS

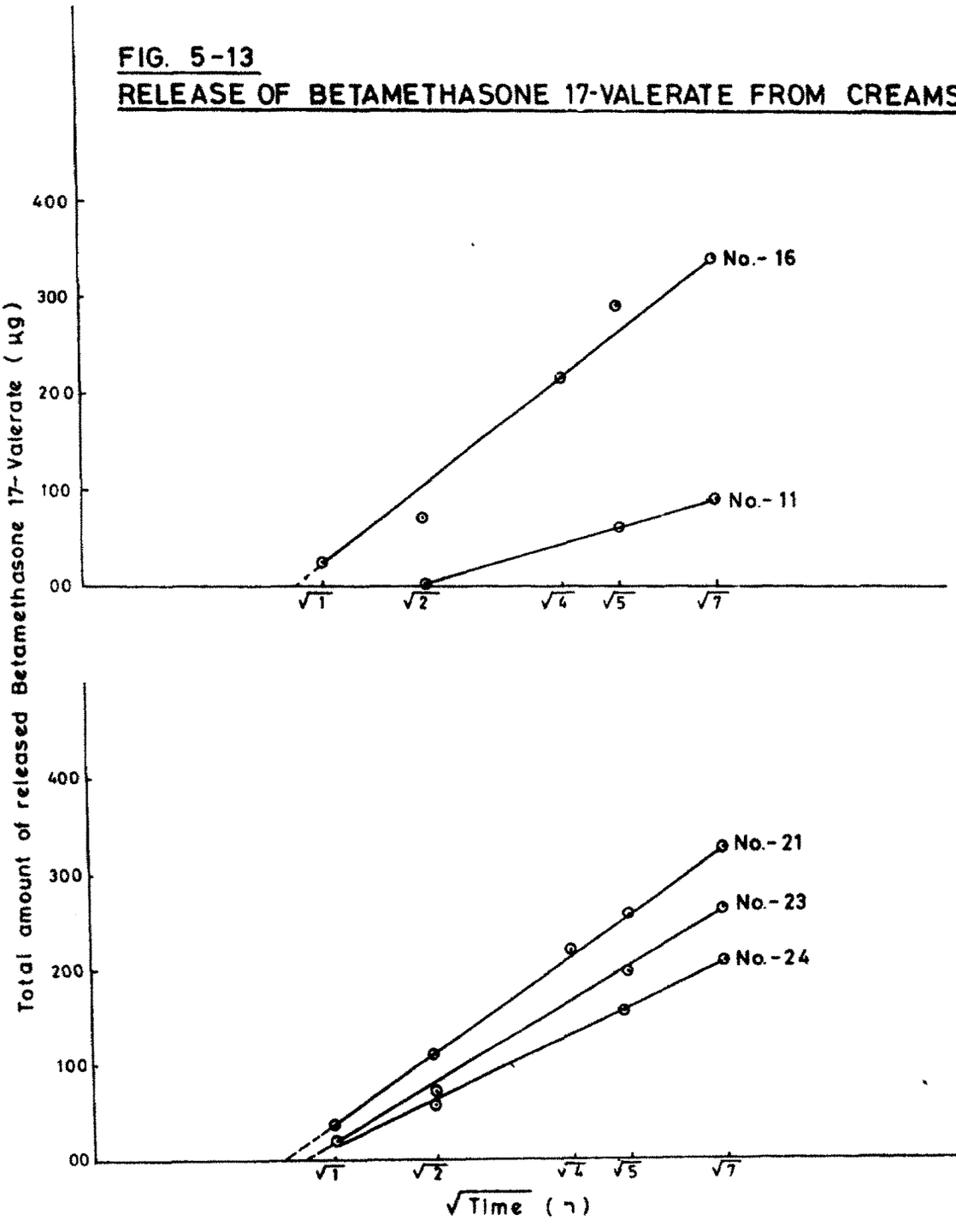


FIG. 5-14

RELEASE OF BETAMETHASONE 17-VALERATE FROM CREAMS

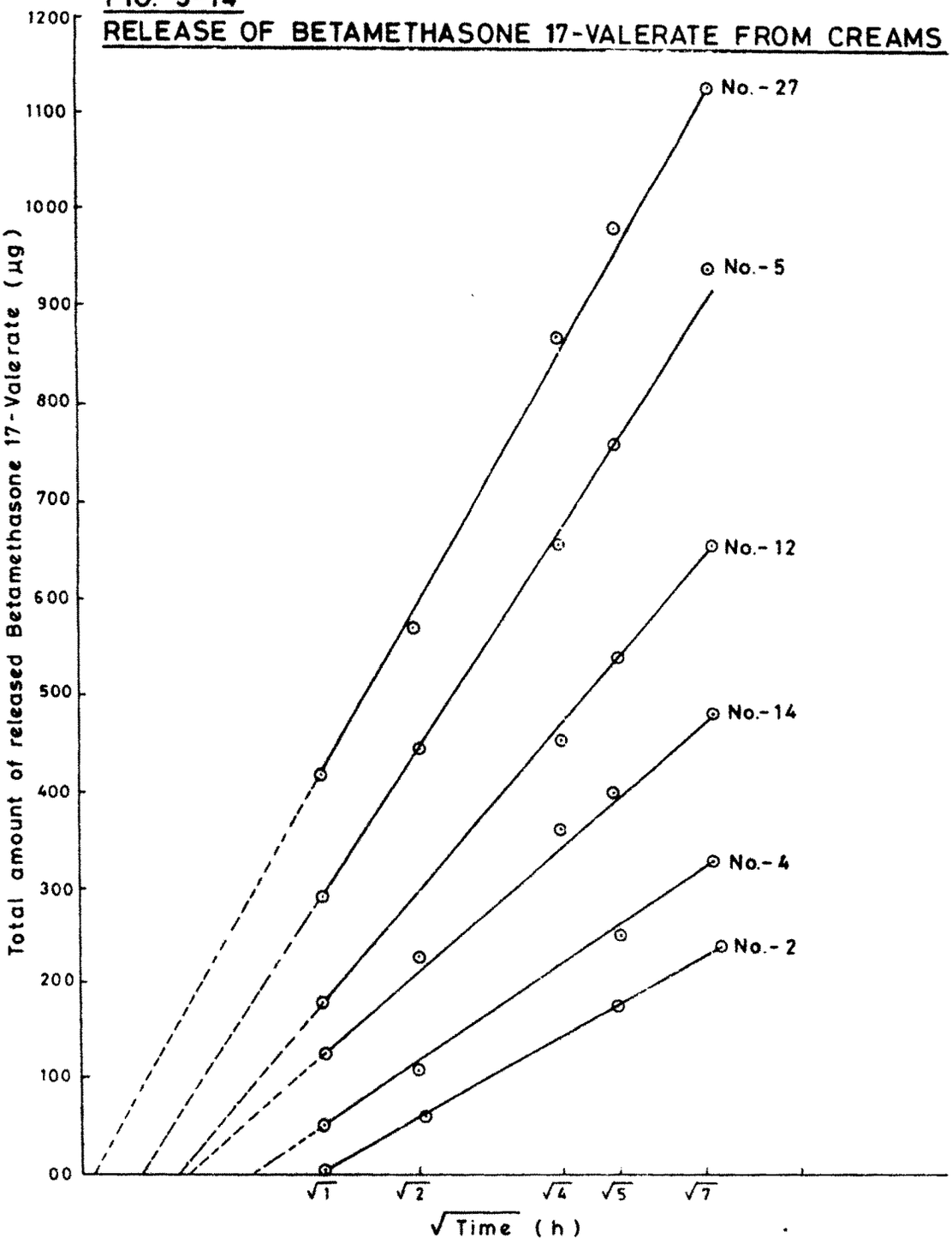


TABLE 3-1 : **Cumulative Percentage Release of Halcinonide from Creams.**

Cream Nos.	Time (hr)	Cumulative Percentage Release*				
		1	2	4	5	7
1		3.59	5.13	8.70	9.94	10.68
2		0.39	1.05	-	2.68	2.99
3		NIL	0.62	2.07	2.66	3.31
4		NIL	0.39	-	1.21	1.44
5		2.12	4.13	6.90	7.70	9.40
6		0.70	1.24	-	2.38	2.73
7		1.25	2.08	3.40	3.92	4.50
8		0.31	1.20	2.46	2.81	3.31
9		NIL	0.39	-	1.13	1.44
10		NIL	0.64	-	2.01	2.46
11		NIL	0.16	-	0.48	1.14
12		0.55	1.38	-	3.02	4.09
13		2.34	3.83	5.67	6.31	7.30
14		0.70	1.17	-	2.22	2.64
15		NIL	0.56	1.23	1.99	2.22
16		0.39	1.06	-	1.66	2.11
17		0.39	1.21	1.68	2.11	2.46
18		NIL	0.39	-	1.13	1.59
19		0.86	1.26	2.38	2.80	3.30
20		NIL	0.41	-	1.52	1.95
21		0.62	1.32	1.92	2.44	2.80
22		NIL	0.56	1.23	1.76	1.98
23		0.55	1.23	1.84	2.28	2.47
24		NIL	0.39	0.91	1.41	1.77
25		2.12	3.58	5.27	5.90	6.89
26		0.70	1.24	1.92	2.26	2.70
27		1.95	3.71	6.29	6.70	8.61

* Average of two readings.

FIG. 5-15
RELEASE OF HALCINONIDE FROM CREAMS

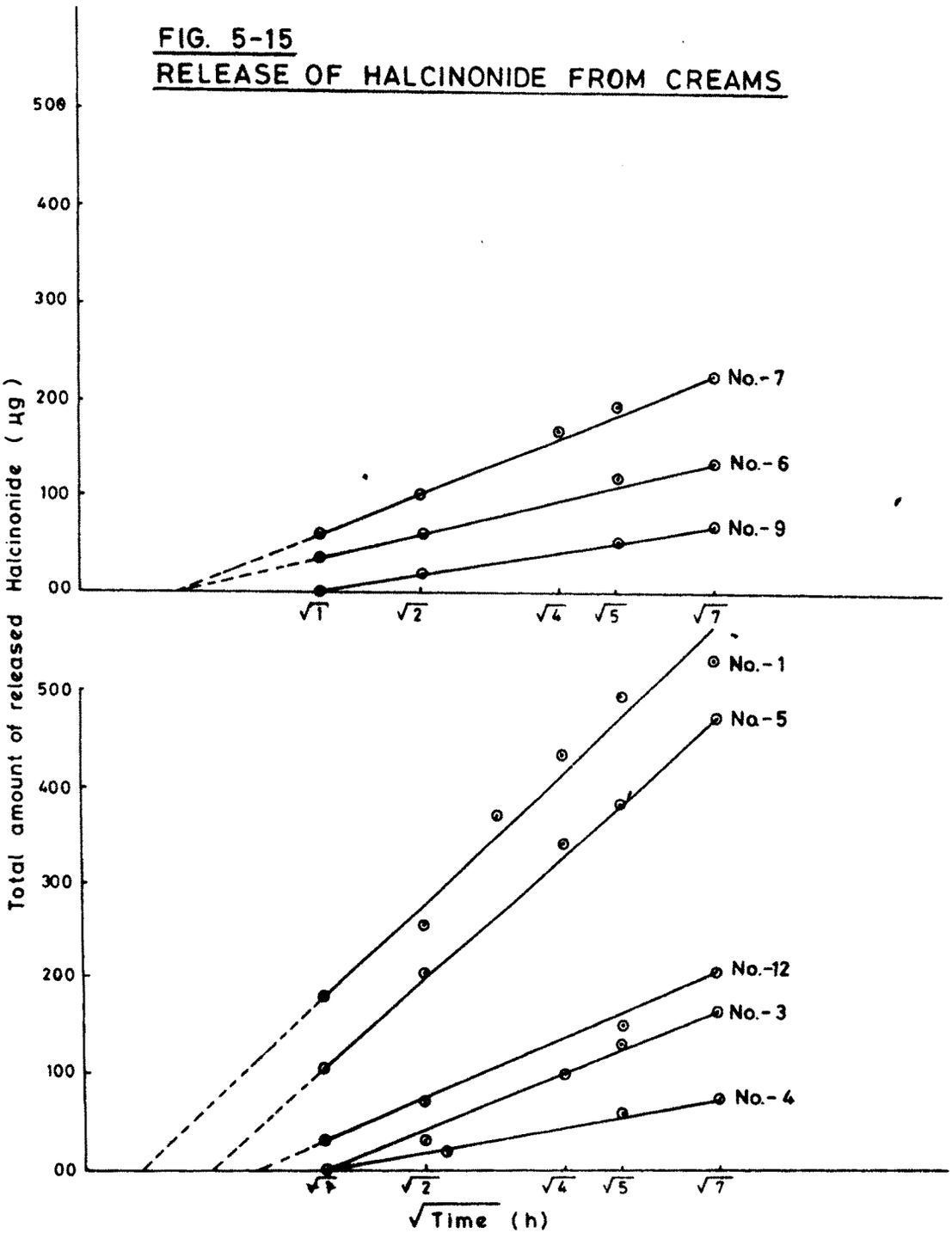


FIG. 5-16
RELEASE OF HALCINONIDE FROM CREAMS

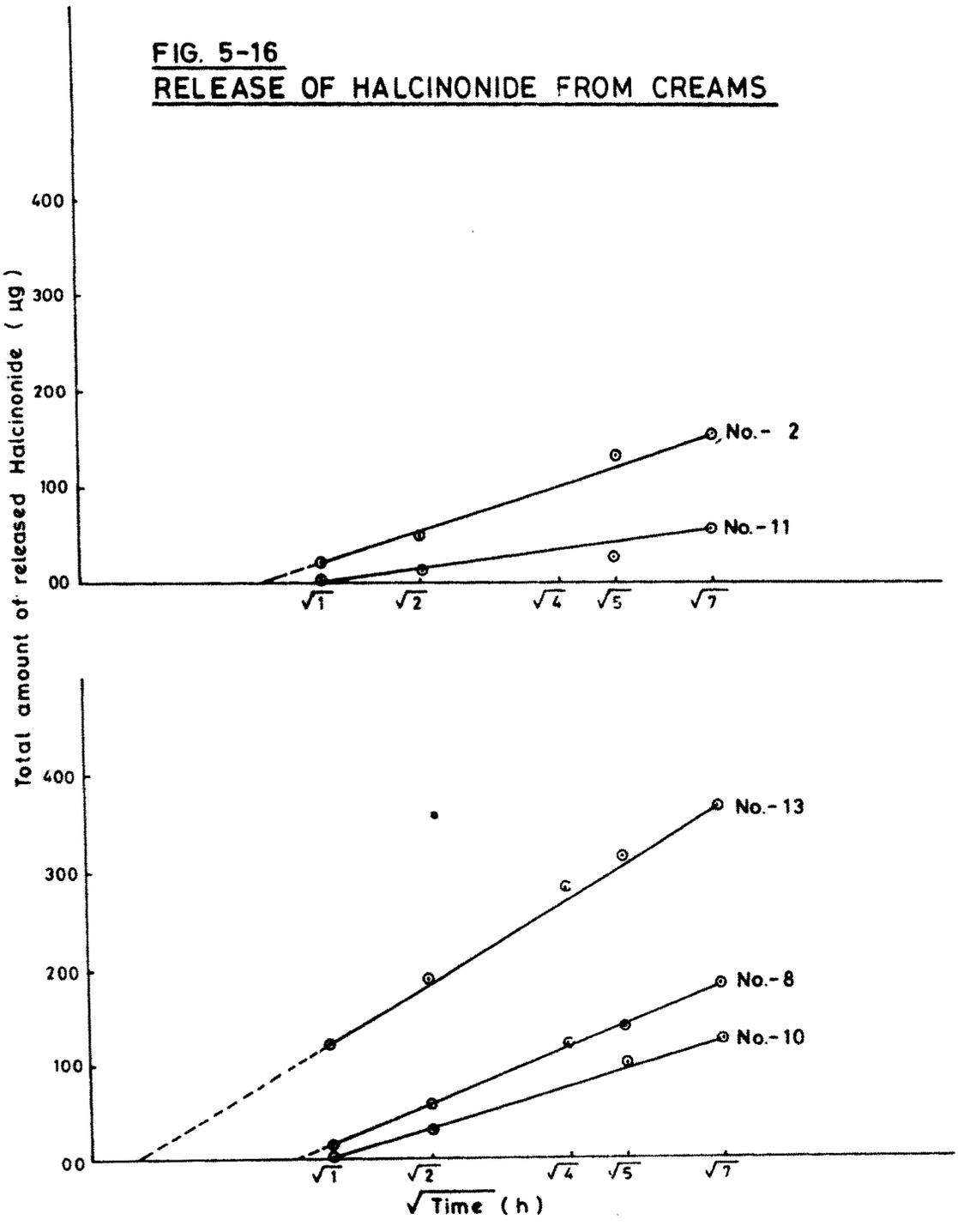


FIG. 5-17
RELEASE OF HALCINONIDE FROM CREAMS

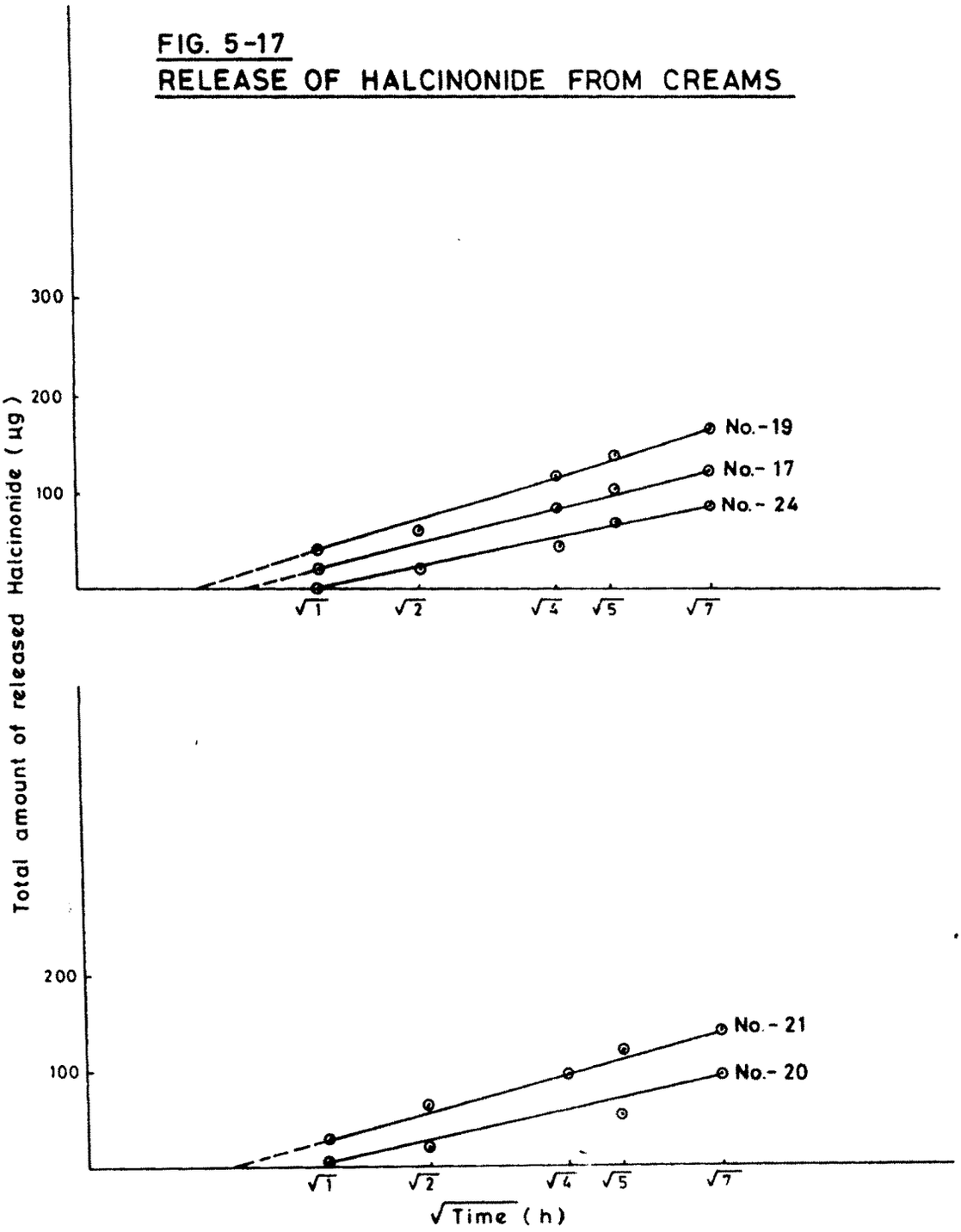


FIG. 5-18
RELEASE OF HALCINONIDE FROM CREAMS

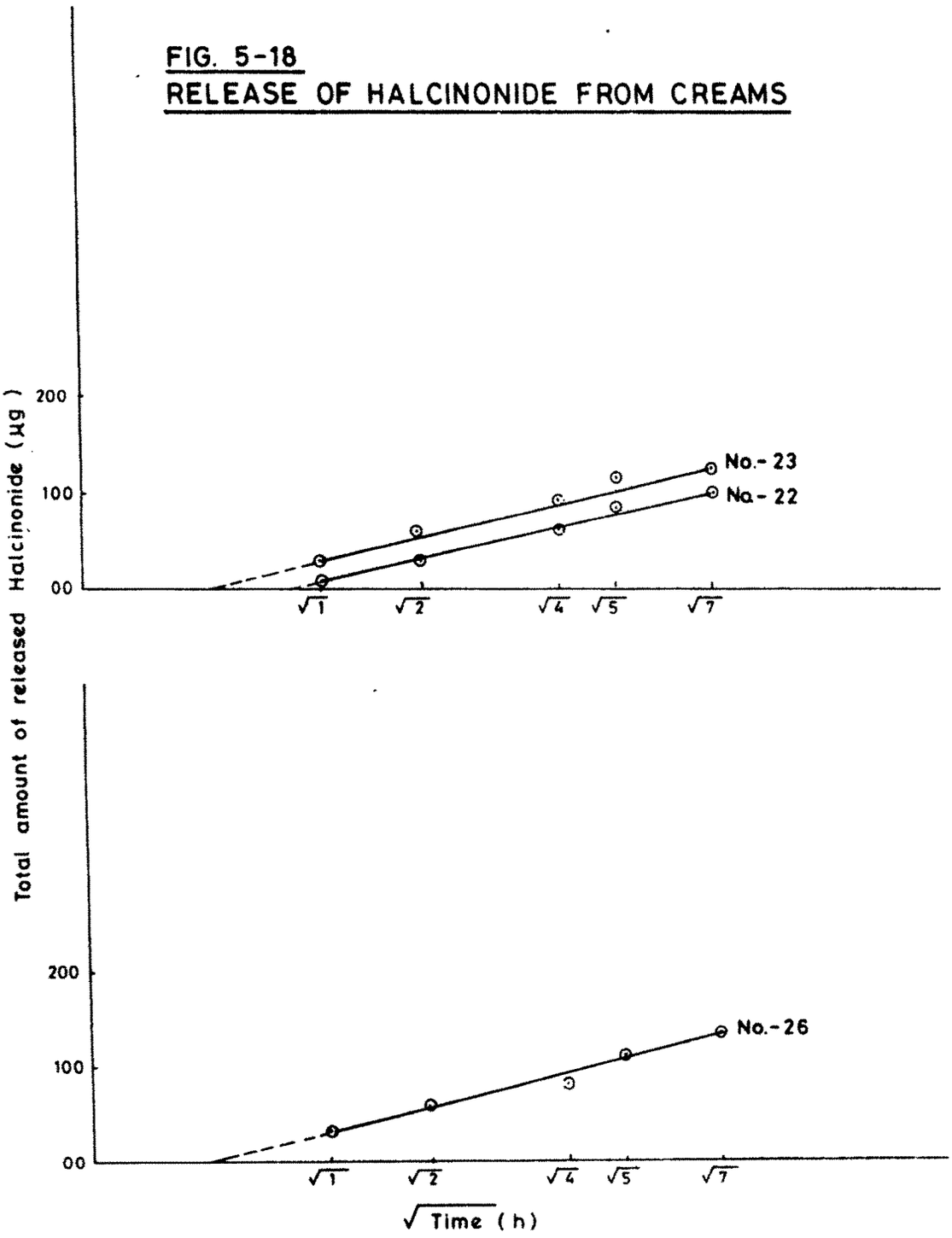


FIG. 5-19
RELEASE OF HALCINONIDE FROM CREAMS

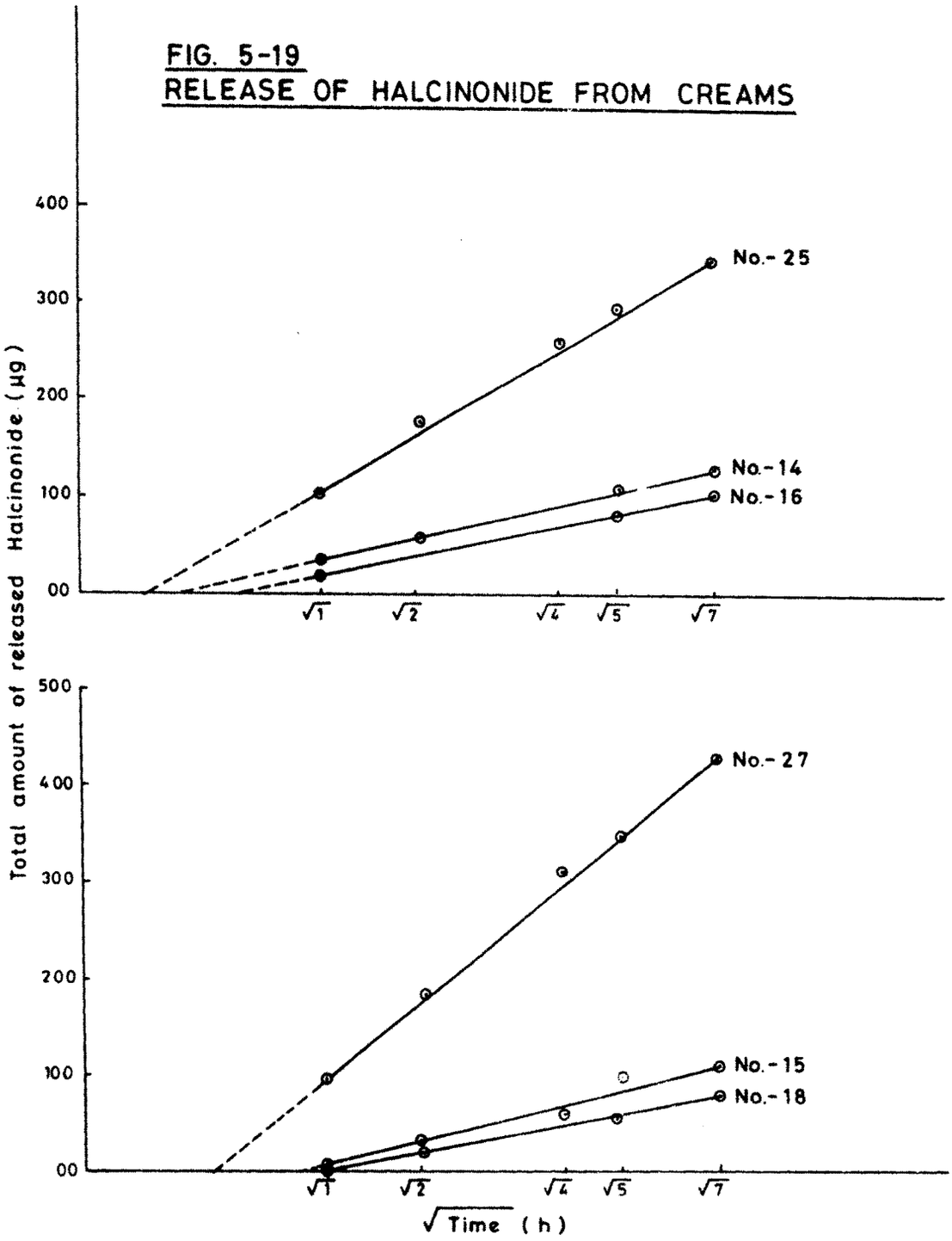


TABLE 5-4 : Cumulative Percentage Release of Fluocinolone Acetonide from Creams.

Cream Nos	Time (hr)	Cumulative Percentage Release*				
		1	2	4	5	7
1		6.68	10.56	17.18	18.03	19.80
2		0.48	1.86	-	5.02	7.38
3		2.36	4.48	7.62	8.50	10.08
4		1.09	3.14	-	7.56	10.41
5		4.29	8.05	12.67	14.52	17.64
6		2.38	5.11	7.88	10.19	12.21
7		4.36	6.33	9.93	11.19	13.99
8		2.83	5.71	8.81	10.98	12.82
9		2.94	5.26	9.35	10.81	12.33
10		NIL	1.10	-	4.11	5.70
11		NIL	0.43	-	1.45	2.30
12		1.82	4.67	9.54	11.21	14.36
13		7.02	10.35	14.65	16.61	19.50
14		0.59	2.54	4.76	5.75	7.60
15		0.71	1.74	-	3.62	4.87
16		NIL	0.98	-	2.94	3.50
17		NIL	1.55	-	3.49	4.75
18		1.67	3.14	-	6.61	8.19
19		3.46	5.35	-	9.80	12.14
20		0.59	1.59	-	4.40	6.08
21		2.59	4.50	7.02	8.91	10.49
22		NIL	1.69	4.65	5.65	7.59
23		1.42	3.21	-	5.97	7.41
24		NIL	1.53	-	3.21	4.20
25		4.48	7.46	11.78	13.25	16.18
26		0.83	2.44	-	4.36	7.49
27		6.67	9.95	14.02	15.48	18.90

* Average of two readings.

FIG. 5-20
RELEASE OF FLUOCINOLONE ACETONIDE FROM CREAMS

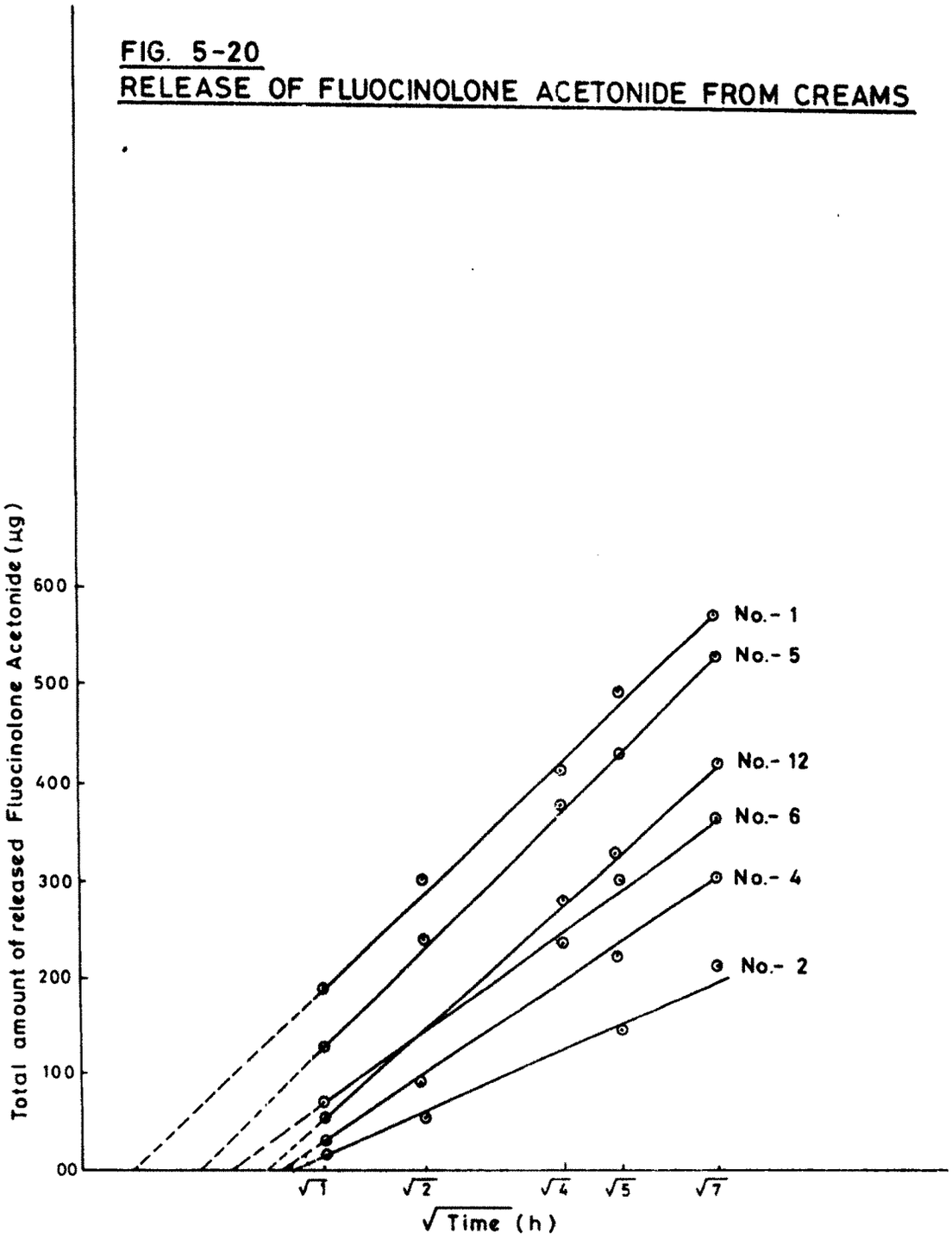


FIG. 5-21
RELEASE OF FLUOCINOLONE ACETONIDE FROM CREAMS

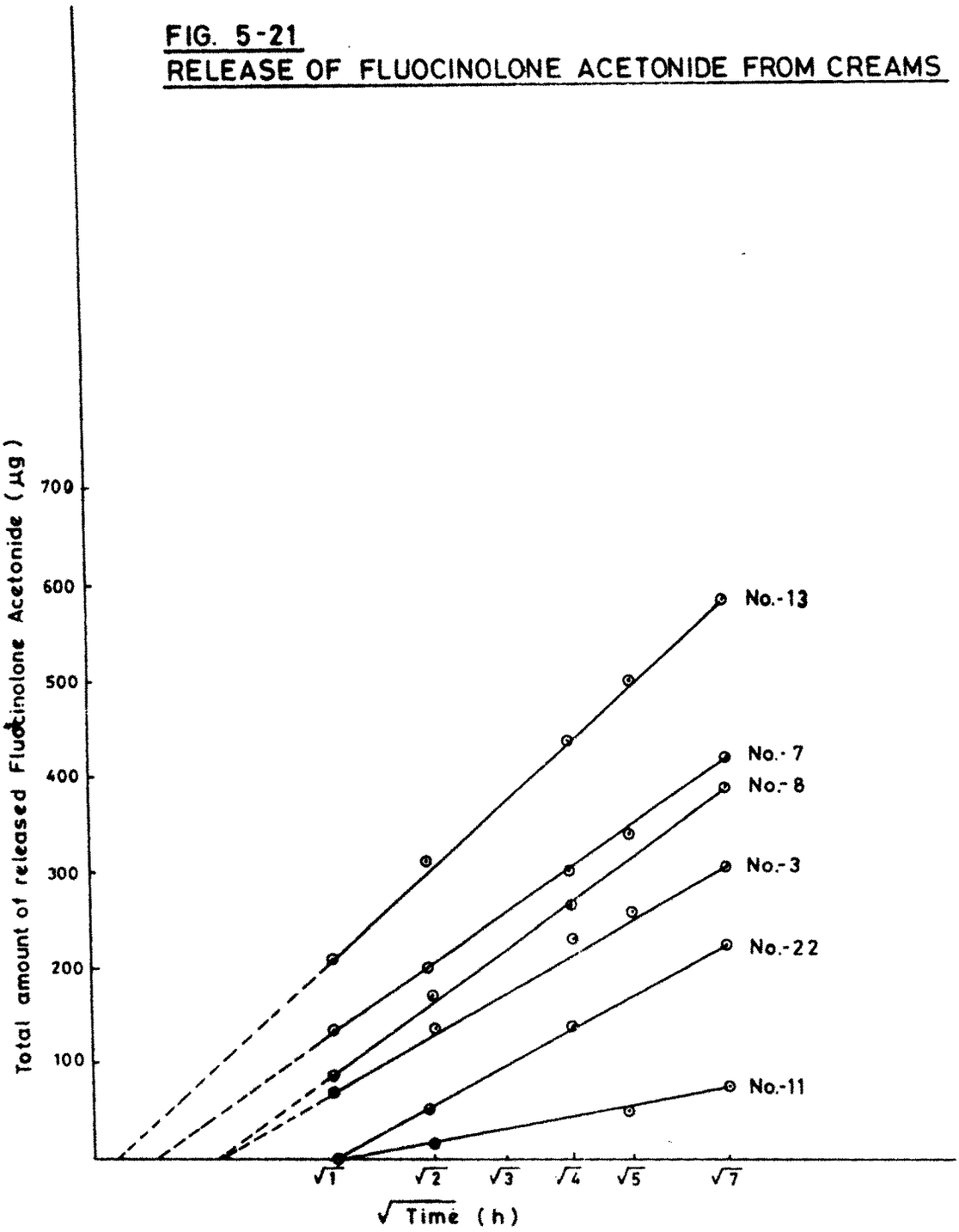


FIG. 5-22
RELEASE OF FLUOCINOLONE ACETONIDE FROM CREAMS

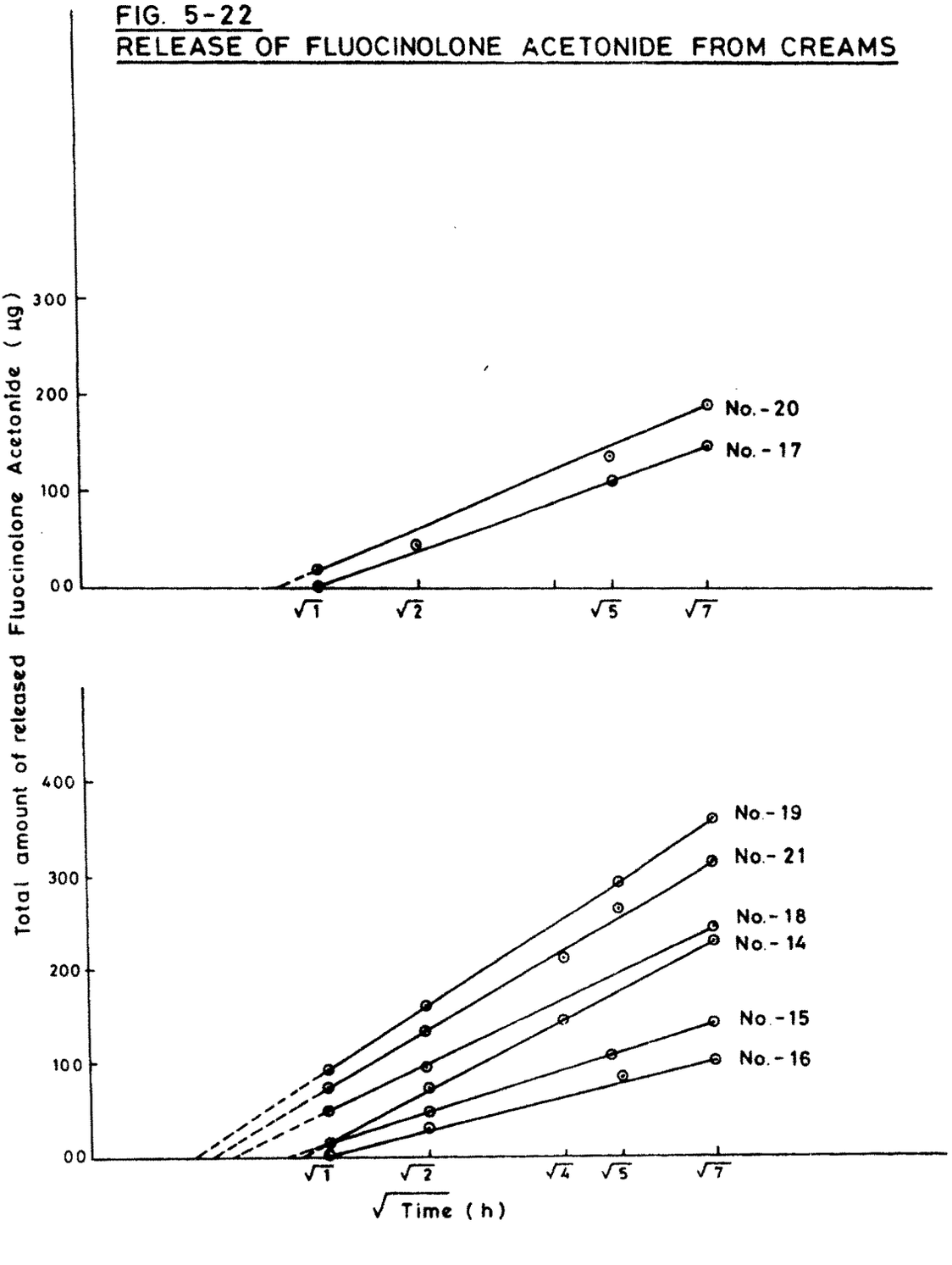
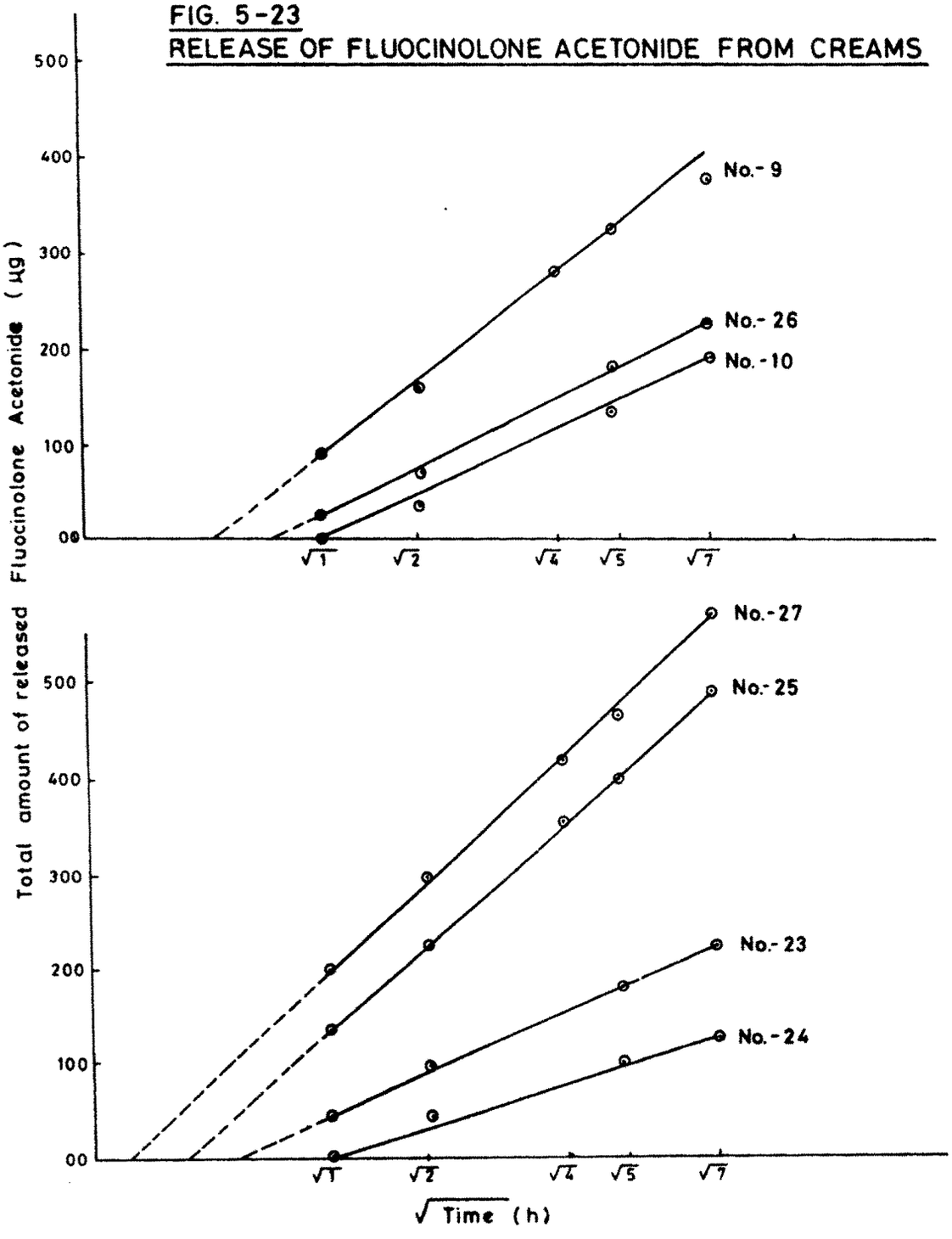


FIG. 5-23
RELEASE OF FLUOCINOLONE ACETONIDE FROM CREAMS



4.6. Drug Releasing Efficiency

One of the concepts introduced by Khan[70] to compare the drug dissolution profiles of different formulations is Drug Releasing Efficiency (DRE). He introduced this new parameter to compare the dissolution efficiency of drugs incorporated in tablets. However, the same concept has been utilised here to compare the drug releasing efficiency of different bases. The application of this concept has been made as follows.

The drug releasing efficiency of the cream base can be defined as the area under the drug release curve upto a certain time 't' expressed as a percentage of the area of the rectangle described by 100% drug released in same time.

$$\text{Drug Releasing Efficiency} = \frac{\text{Shaded area}}{\text{Rectangle } (CR_{100} \times Ot)} \times \frac{100}{1}$$

This concept of drug releasing efficiency has certain advantage such as it gives summation of the drug release data into a single figure which provides a means for reading comparison of a large number of formulations.

The DRE of the prepared cream was calculated at time t = 7 hr further the area under drug release curve was measured by counting the squares below the curve, full or in parts and adding them together. For DRE each curve was drawn separately and carefully the

TABLE 5-6 : Release Rate from the Selected Bases Containing Drugs.

Cream Nos.	TA mg/√h	BV mg/√h	HAL mg/√h	FA mg/√h
1.	385	590	230	235
2.	170	140	60	110
3.	180	315	100	140
4.	87	170	45	170
5.	295	390	220	243
6.	185	280	105	177
7.	215	305	102	175
8.	210	285	105	185
9.	190	115	40	150
10.	120	185	75	110
11.	55	75	33	45
12.	172	305	80	220
13.	375	420	150	230
14.	157	220	55	130
15.	122	70	63	77
16.	125	190	50	60
17.	150	210	62	85
18.	152	190	50	118
19.	185	200	76	160
20.	122	195	54	100
21.	140	180	65	145
22.	180	100	58	135
23.	145	148	60	100
24.	142	125	52	75
25.	300	290	145	205
26.	140	180	60	120
27.	395	430	203	220

squares under the curve were counted. The drug release efficiency expressed on percentage basis for all the creams are recorded in Table 5-5.

After comparing the data of *in vitro* release studies, selected creams subjected to further study for the liberation of corticosteroid from creams through sartorius ointment chamber assembly.

4.7. Liberation of Active Ingredients from Creams through Sartorius Ointment Chamber Assembly :

Sartorius has developed an *in vitro* liberation unit for examining the liberation of active ingredients from ointments. This ointment chamber unit having a system in which active ingredients can diffuse from an ointment base through a double-layer membrane into a liquid acceptor medium. This membrane has been specially designed to allow all types of ointment bases to be tested, independent of their composition and their physical characteristics.

The sartorius ointment chamber assembly (ointment chamber complete with pump and temperature regulating system) is a valuable aid to the pharmaceutical and cosmetic industry in testing ointments, since it is practically impossible to carry out all the necessary tests for active ingredient liberation *in vivo* on human skin.

4.7.a. Description of the Apparatus :

The sartorius ointment chamber assembly consists principally of the following parts.

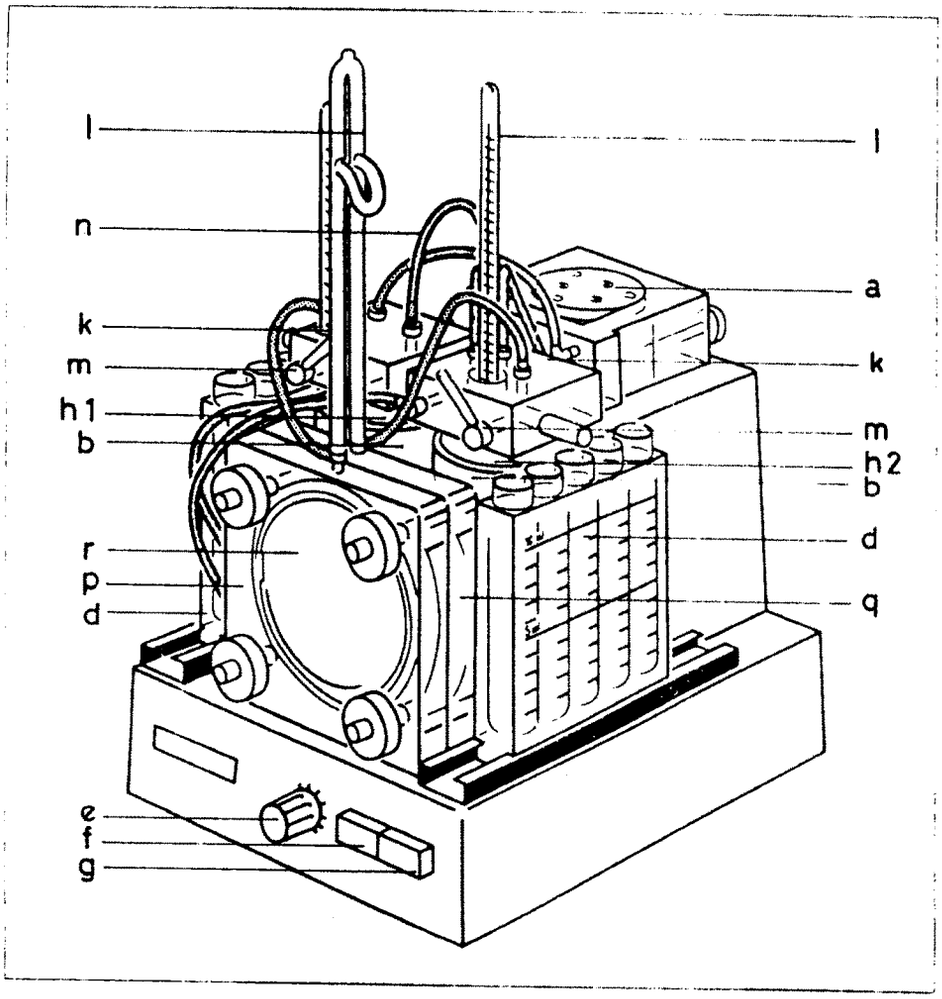
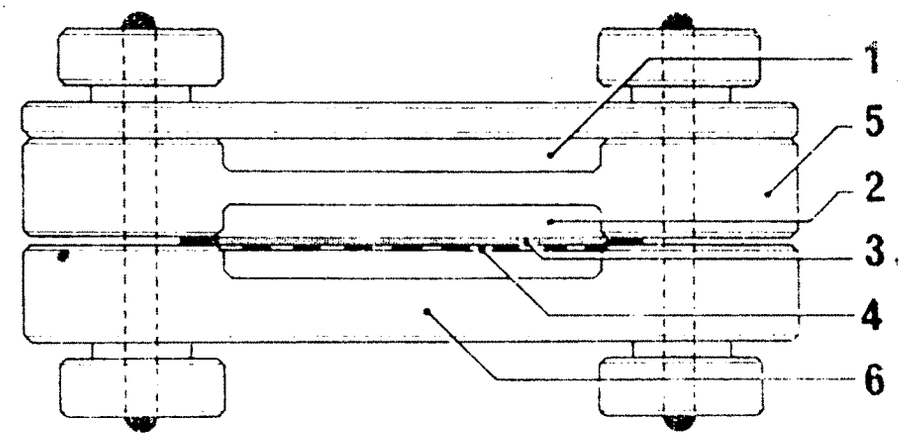


FIG. 6-1 : Sartorius Ointment Chamber Assembly.



1 Temperature regulating space · 2 Ointment · 3 Double-layer membrane · 4 Corrugated plate · 5 Middle plate · 6 Bottom plate

FIG. 6-2 : Cross Section of the Sartorius Ointment Chamber.

4.7.a.1. Basic instrument :

Peristaltic Pump (a) to circulate the aqueous phases.

Temperature Jacket (b) for the containers h_1 and h_2.

Stirrer motor (not visible) (c) for the magnetic stirrer bars.

Moveable holder (d) for test tubes.

Control knob (e) for temperature adjustment.

Left-hand push button (f) on-off switch for the stirrer motor and pump.

Right-hand push button (g) switch for heating and pumping direction of pump.

4.7.a.2. The containers h_1 and h_2 for the aqueous phases 1 & 2 :

Magnetic stirrer

Distribution cap (k)

Thermometer (l)

Taps (M) ---------- for sampling.

Tube connections (n).

4.7.a.3. The diffusion chamber (Type A)

Front plate (P)

Back plate (q)

Lipid barrier (r)

The sartorius ointment chamber consisted of three transparent plexiglass plates which were held together by bolts and nuts. Two of these plates had central cavities as shown in the Figure 6-2. Water, at a temperature of 37° was circulated at a flow rate of 13 ml/min, through out the temperature regulating space (1) of the middle plate (5). This plate (5)

also had a well (2) on the opposite side in which 6.0 g of cream was placed. In plate (6), the acceptor medium was circulated through the corrugated plate (4) across the bottom surface of the membrane (3), and any ingredient which was liberated absorbed by the acceptor medium.

The artificial double-layer membrane (3) was composed of two layers :

- a barrier foil
  soaked in water until swollen.
- a membrane filter, type RS, impregnated with lauryl alcohol.

These layers were pressed together to form a hydrophilic/lipophilic double-layer which was impermeable to the ointment base as well as to the acceptor medium. Molecules of active ingredients, which had been liberated from the ointment, might diffuse through this membrane to reach the medium.

4.7.b. Experimental procedure :

A magnetic stirring bar was placed in each of the containers in the thermostated holders. About 100 ml of distilled water (pH = 6.8) was taken in two containers 'h_1' and 'h_2'. The distributor caps were put on the containers (sampling taps closed) and the following connections were made with the tubing provided : 1-1, 2-2, 3-3, 4-4, 5-7, 6-8. The right hand and left hand push buttons were pressed. The temperature ($39° \pm 1°$) of the aqueous solutions in each of the containers was adjusted with the heating control knob.

The lipid barrier was prepared, as described earlier. Even slight mechanical damage to the barrier is to be avoided, hence the filter should always be handled with special forceps. The prepared membrane was placed in position between the plates of the diffusion chamber, and the plates were screwed together.

When the required temperature was attained, the right hand push button was again pressed so that the tubing was emptied. The pump was switched off by pressing the left hand push button. The diffusion chamber was connected to the distribution caps by connecting the following opening with tubing.
For chamber 8 (effective area 16 cm^2) :

6-5, 8-7, 7-8, 5-6.

After the above connections were made, the right hand and left hand buttons were pressed to start the experiment.

At a suitable intervals, 20 ml of the sample solution was withdrawn from the h_2 container. The drug in the sample extracted thrice with 10 ml of chloroform each time. For the better separation of the layer 1 g of anhydrous sodium phosphate was added during extraction. About 25 ml of chloroform layer was evaporated on water bath at 65 to 70°. Remaining amount of chloroform was allowed to cool at room temperature and analysed for corticosteroid drugs colorimetrically.

TABLE 6-1 : Liberation of Triamcinolone Acetonide from Creams through Sartorius Ointment Chamber Assembly.

Cream Nos.	Time (hr)	Cumulative Percentage Release*			
		1	2	4	7
1		0.17	1.69	4.13	7.19
8		0.12	1.26	3.70	5.33
9		0.07	0.95	1.74	3.07
12		NIL	0.12	0.44	0.82
13		0.37	0.94	3.00	4.56
25		0.12	0.74	2.24	3.48
27		0.27	1.66	4.09	6.70

TABLE 6-2 : Liberation of Betamethasone 17-Valerate from Creams through Sartorius Ointment Chamber Assembly.

Cream Nos	Time (hr)	Cumulative Percentage Release*			
		1	2	4	7
1		0.26	1.36	3.37	5.55
5		0.37	0.90	2.19	3.48
7		0.20	0.75	1.43	2.40
12		NIL	NIL	0.21	0.61
13		0.14	0.85	1.76	2.74
25		0.48	0.75	0.96	1.59
27		0.20	1.44	2.94	4.22

* Average of two readings.

FIG. 6-3 LIBERATION OF TRIAMCINOLONE ACETONIDE FROM CREAMS THROUGH SARTORIUS OINTMENT CHAMBER ASSEMBLY

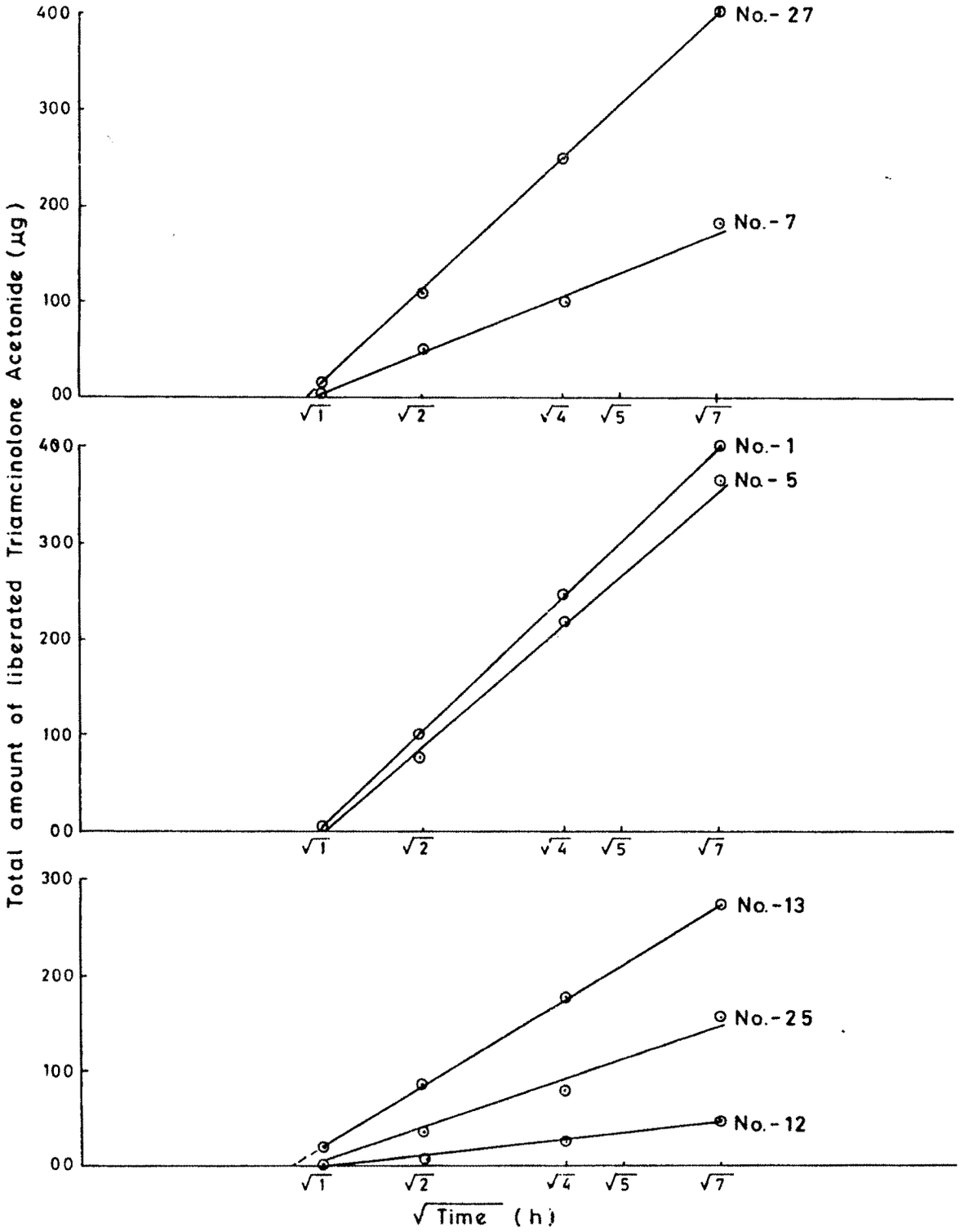


FIG. 6-4 LIBERATION OF BETAMETHASONE 17-VALERATE THROUGH SARTORIUS OINTMENT CHAMBER ASSEMBLY

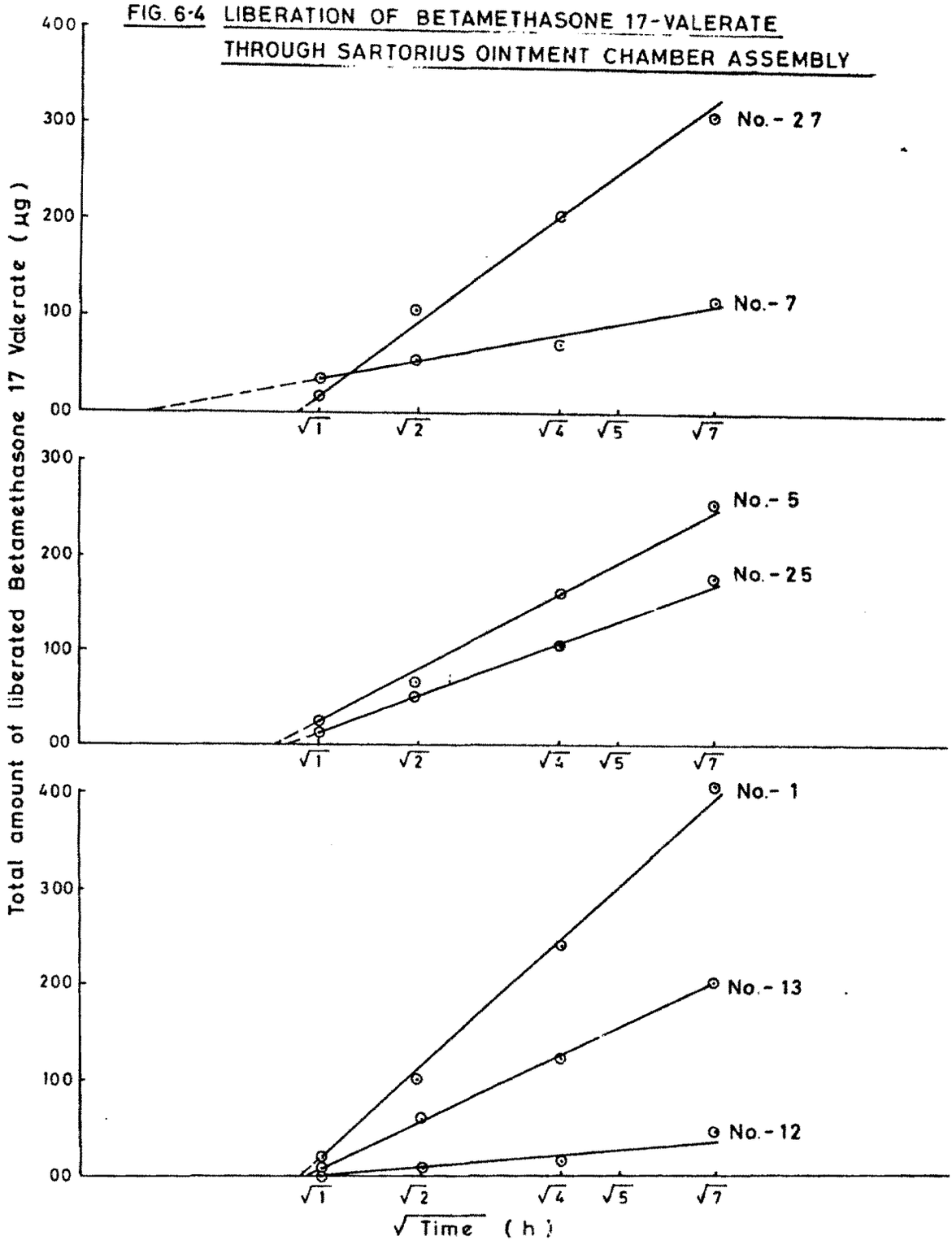


TABLE 6-3 : Liberation of Halcinonide from Creams through Sartorius Ointment Chamber Assembly.

Cream Nos.	Time (hr)	Cumulative Percentage Release*			
		1	2	4	7
1		0.36	1.47	3.47	4.51
3		0.59	1.35	2.85	3.84
7		0.07	0.57	1.35	2.17
12		NIL	NIL	0.27	0.87
13		0.23	1.02	2.34	3.42
25		0.07	0.82	2.21	3.19
27		0.42	1.29	3.01	4.88

TABLE 6-4 : Liberation of Fluocinolone Acetonide from Creams through Sartorius Ointment Chamber Assembly.

Cream Nos.	Time (hr)	Cumulative Percentage Release*			
		1	2	4	7
1		1.31	3.12	4.50	6.14
5		NIL	0.48	1.41	3.60
7		NIL	0.24	0.76	1.33
12		NIL	0.24	0.95	1.21
13		NIL	0.24	0.88	2.07
25		0.24	0.64	1.67	2.33
27		0.83	1.95	3.33	5.01

* Average of two readings.

FIG. 6-5 LIBERATION OF HALCINONIDE FROM CREAMS THROUGH SARTORIUS OINTMENT CHAMBER ASSEMBLY

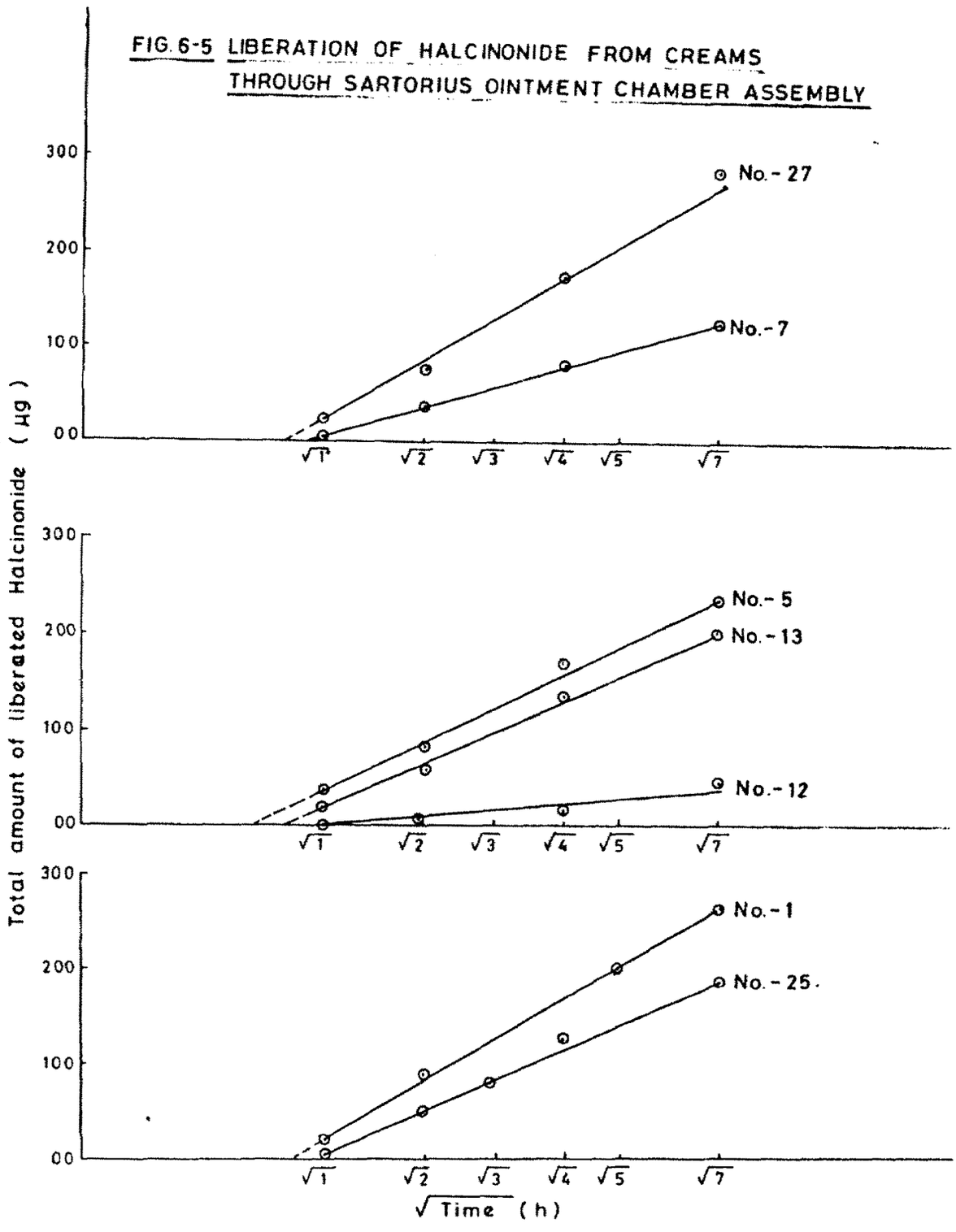
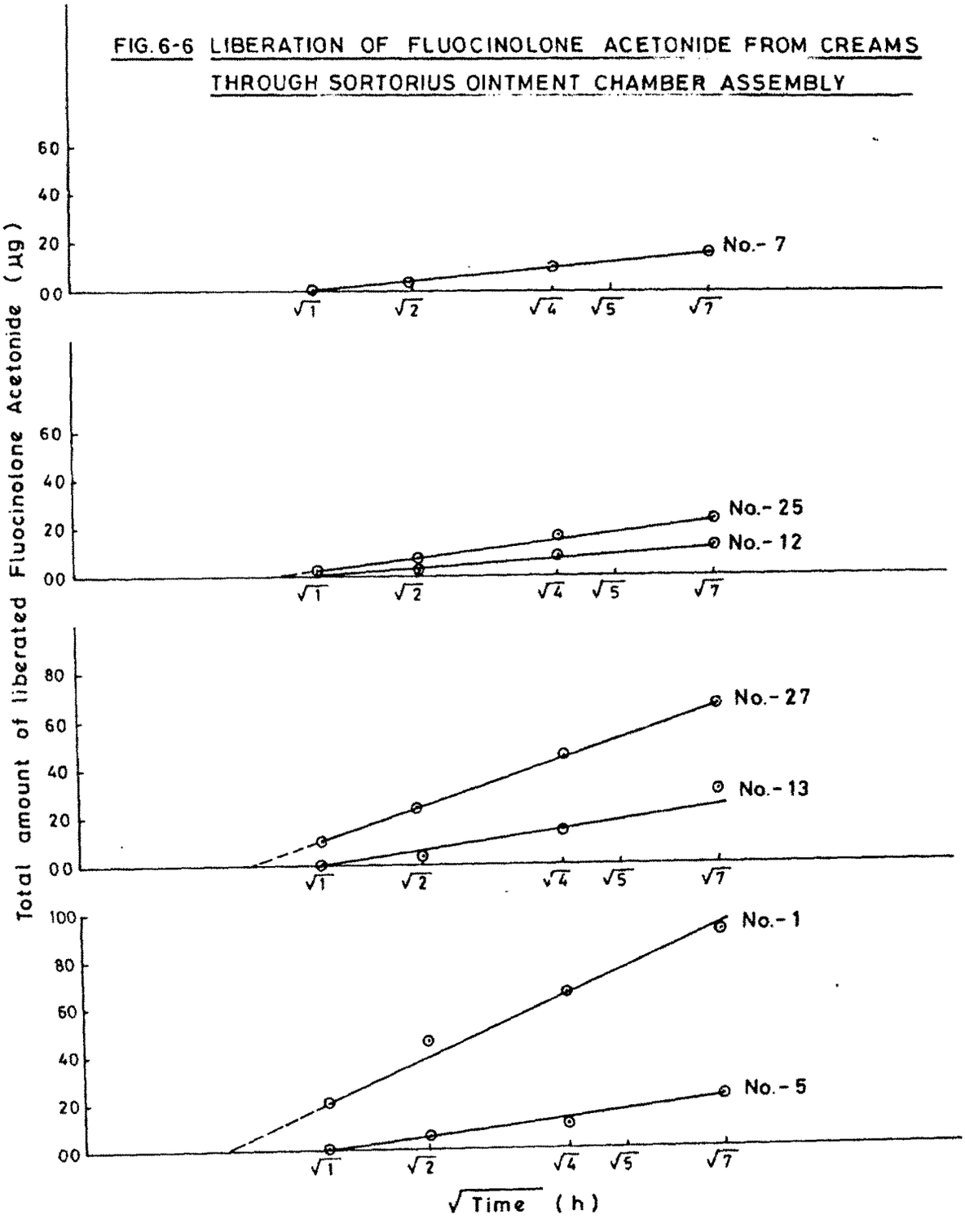


FIG. 6-6 LIBERATION OF FLUOCINOLONE ACETONIDE FROM CREAMS THROUGH SORTORIUS OINTMENT CHAMBER ASSEMBLY



4.8. Results and Discussion :

(a) Triamcinolone acetonide creams:

It can be observed from the data in Table 5-1 and fugures 5-2 to 5-8 that the hydrophilic cream No. 27, containing cetomacrogol 1000, cetostearyl alcohol, polyoxyethylene stearate, polyethylene glycol 6000 distearate, light liquid paraffin, propylene glycol and 2-pyrrolidone is the best in its cumulative percentage release of the medicament. The cream bases based on the medicament release in decreasing order could be arranged as follows.

Cream No. 27 > 1 > 13 > 5 > 25 > 7 > 8 > 12 > 9 >
6 > 19 > 2 > 3 > 14 > 22 > 26 > 23 > 17 >
16 > 18 > 21 > 24 > 10 > 15 > 20 > 4 > 11.

(b) Betamethasone 17-valerate creams :

In case of betamethasone 17-valerate creams it can be observed from the data in Table 5-2 and figures 5-9 to 5-14 that the hydrophilic cream No. 1, containing Glyceryl monostearate S.E., stearyl alcohol, cetomacrogol 1000, Isopropyl-myristate, light liquid paraffin, glycerin and castor oil is the best in its cumulative percentage release of the medicament. The cream bases based on the medicament release in decreasing order could be arranged as follows.

Cream No. 1 > 27 > 13 > 5 > 7 > 3 > 12 > 25 >
6 > 8 > 14 > 17 > 10 > 19 > 4 > 20 >
16 > 18 > 26 > 21 > 23 > 22 > 2 > 9 >
24 > 11 > 15.

(c) Halcinonide creams :

In case of halcinonide creams, (Table 5-3 and Figures 5-15 to 5-19) the hydrophilic cream base No. 1 containing glyceryl monostearate S.E., Stearyl alcohol, Cetomacrogol 1000, Isopropyl myristate, light liquid paraffin, glycerin and castor oil is the best in its cumulative percentage release of the medicament. The cream bases based on the medicament release in decreasing order could be arranged as follows.

Cream No. 1 > 5 > 27 > 13 > 28 > 7 > 12 > 3 & 8 >
19 > 2 > 21 > 6 > 26 > 14 > 23 > 10 & 17 >
15 > 16 > 22 > 20 > 24 > 18 > 4 & 9 > 11.

(d) Fluocinolone acetonide creams :

In case of fluocinolone acetonide creams (Table 5 and Figures 5-20 to 5-23) the hydrophilic cream base No. 1 is the best in its cumulative percentage release of the medicament. The cream bases based on the medicament release in decreasing order could be arranged as follows.

Cream No. 1 > 13 > 27 > 5 > 25 > 12 > 7 > 8 >
9 > 6 > 19 > 21 > 4 > 3 > 18 > 14 >
22 > 26 > 23 > 2 > 20 > 10 > 15 > 17 >
24 > 16 > 11.

It can be observed from the data recorded in Tables 5-1 to 5-4 and represented in figures 5-2 to 5-23 that there is no fixed pattern of release from all the creams containing different drugs. However in some of the creams there is initially some lag periods as far as the release is concerned.

It can be observed from the data that Cream No. 15 containing white soft paraffin, cetyl alcohol, cetomacrogol emulsifying wax, heavy liquid paraffin and borax, has shown minimum release of betamethasone 17-valerate from betamethasone 17-valerate cream.

It can be observed from the data that Cream No. 11 containing glyceryl monostearate, stearic acid and white soft paraffin has shown minimum release of triamcinolone acetonide, halcinonide and fluocinolone acetonide from respective creams.

It is further notable that the cumulative percent release of the all the four drugs is less from the cream bases containing white soft paraffin, white bees wax alone or in combinations.

It has been reported that the optimum release of medicament was obtained when the surfactants (spans and tweens) were employed in 1% concentration so in cream No. 25, Tween 80 was used and cream showed the total release of 14.71% of triamcinolone acetonide, 9.75% of

betamethasone 17-valerate, 6.89% of halcinonide and 16.18% of fluocinolone acetonide in 7 hr. HLB value of Tween 80 is 15 and lies within the range of 8-18 (o/w emulsification), and 15-18 (solubilising agents), possibly it may be acting as o/w emulsifying agent and solubilizing agent in these formulations.

The drug releasing efficiency of the cream bases were found out and represented in the Table 5-5. The DRE data of the cream bases followed the following decreasing order for

(e) Triamcinolone acetonide creams

27 > 1 > 13 > 5 > 25 > 7 > 9 > 8 > 19 > 6 > 12 > 3 > 26 > 2 > 14 > 23 > 22 > 16 > 17 > 21 > 18 > 24 > 20 > 15 > 10 > 4 > 11.

(f) Betamethasone 17-valerate creams

1 > 27 > 13 > 5 > 7 > 3 > 12 > 25 > 6 > 8 > 14 > 10 > 19 > 17 > 21 > 4 > 16 > 20 > 18 > 22 > 26 > 9 > 23 > 2 > 24 > 11 > 15.

(g) Halcinonide creams

1 > 5 > 27 > 13 > 25 > 7 > 12 > 6 > 19 > 8 > 21 > 2 > 26 > 14 > 3 > 17 > 23 > 10 > 16 > 15 > 22 > 20 > 24 > 4 > 9 > 18 > 11.

(h) Fluocinolone acetonide creams

1 > 13 > 27 > 5 > 25 > 7 > 8 > 12 > 9 > 19 > 6 > 21 > 3 > 4 > 18 > 23 > 26 > 22 > 14 > 2 > 20 > 10 > 15 > 17 > 24 > 16 > 11.

It is known that the amount of drug released from the formulation is approximately proportional to the square root of time. When the amount of triamcinolone acetonide, betamethasone 17-valerate, halcinonide and fluocinolone acetonide released from the respective creams were plotted versus $\sqrt{\text{time}}$ in hr., straight lines were obtained in almost all the creams. The release rate $\mu g/\sqrt{\text{time}}$ was calculated from the slope of the straight line obtained.

The release rate of the cream bases were found out and the data recorded in Table 5-6 and represented in figures 5-2 to 5-23. The release rate of the cream bases followed the following decreasing order for

(i) <u>Triamcinolone acetonide creams</u>

27 > 1 > 13 > 25 > 5 > 7 > 8 > 9 > 6 & 19 > 22 & 3 > 12 > 2 > 14 > 18 > 17 > 23 > 24 > 26 & 21 > 16 > 20 & 15 > 10 > 4 > 11.

(j) <u>Betamethasone 17-valerate creams</u>

1 > 27 > 13 > 5 > 3 > 7 & 12 > 25 > 8 > 6 > 14 > 17 > 19 > 20 > 18 & 16 > 10 > 21 & 26 > 4 > 23 > 2 > 24 > 9 > 22 > 11 > 15.

(k) <u>Halcinonide creams</u>

1 > 5 > 27 > 13 > 25 > 6 & 8 > 7 > 3 > 12 > 19 > 10 > 21 > 15 > 17 > 2, 26 & 23 > 22 > 14 > 20 > 24 > 16 & 18 > 4 > 9 > 11.

(l) <u>Fluocinolone acetonide creams</u>

5 > 1 > 13 > 12 & 27 > 25 > 8 > 6 > 7 > 4 > 19 > 9 > 21 > 3 > 22 > 14 > 26 > 18 > 2 & 10 > 20 & 23 > 17 > 15 > 24 > 16 > 11.

After comparing the data of cumulative percentage release, drug releasing efficiency and Release rate of all selected formulations, cream Nos. 1,5,7,12,13,25, 27 were selected for further study of liberation of corticosteroid from selected creams through sartorius ointment chamber assembly, as they have proved to be superior in comparison to other creams.

The cumulative percentage release of corticosteroids from respective creams through Sartorius Ointment Chamber assembly is as under.

(m) Triamcinolone acetonide

It can be observed from the data recorded in Table 6-1 and represented in Figure 6-3 that hydrophilic cream No. 1 is the best in its cumulative percentage release of triamcinolone acetonide from cream out of seven selected creams after *in vitro* evaluation in laboratory model. The cream bases based on the medicament release in decreasing order could be arranged as follows.

1 > 27 > 5 > 13 > 25 > 7 > 12.

(n) Betamethasone 17-valerate

It can be observed from the data recorded in Table 6-2 and represented in Figure 6-4 that hydrophilic cream No. 1 is the best in its cumulative percentage release of betamethasone 17-valerate from cream out of seven selected creams after *in vitro* evaluation in laboratory model. The cream bases based

on the medicament release could be arranged as follows.

1 > 27 > 5 > 13 > 7 > 25 > 12.

(o) Halcinonide creams

It can be observed from the data recorded in Table 6-3 and represented in Figure 6-5 that hydrophilic cream No. 1 is the best in its cumulative percentage release of halcinonide from cream. The cream bases based on the medicament release could be arranged as follows.

1 > 27 > 5 > 13 > 25 > 7 > 12.

(p) Fluocinolone acetonide creams

It can be observed from the data recorded in Table 6-4 and represented in Figure 6-6 that hydrophilic cream No. 1 is the best in its cumulative percentage release of fluocinolone acetonide from cream. The cream bases based on the medicament release could be arranged as follows.

1 > 27 > 5 > 25 > 13 > 7 > 12.

The amount of triamcinolone acetonide, betamethasone 17-valerate, halcinonide and fluocinolone acetonide released from the respective creams were plotted versus time in hr. Straight lines were obtained in almost all the creams.

Thus from this part of the wrok it could be concluded that liberation of corticosteroids through sartorius ointment chamber assembly, showed nearly same pattern of release as found in

in vitro release study in laberatory designed model but lesser in amount.

After comparing the data of all the selected TA, HAL and FA, formulations, cream Nos. 1, 5, 13, 25, 27 were selected for further study of bioavailability, employing vasoconstricter assay. The BV Cream Nos. 1, 5, 7, 13, 27 were selected for bioavailability, employing vasoconstricter assay.

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